ARCHIVES OF PATHOLOGY

VOLUME 29 JUNE 1940 NUMBER 6

EXPERIMENTALLY INDUCED BENIGNANCY OF NEOPLASM

IV. SUPPRESSION OF MITOTIC ACTIVITY BY SO-CALLED IMMUNIZATION

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In studies conducted over twenty-five years ago on the inhibition of malignant growth produced by so-called immunization,[1] Woglom[2] had the impression that sarcoma so inhibited showed fewer mitoses in the histologic section than did the rapidly growing controls. He assumed that such momentary estimation of the number of mitoses measured the actual rate of the mitotic multiplication of cells going on in the tissue. This assumption was not checked by direct quantitative observations.

In recent years Dustin,[3] Allen and his co-workers[4] and Brues and Cohen[5] have introduced, in histologic studies, the use of colchicine to exaggerate the evidence of mitotic division. Although various investigators disagree at the moment on the mechanism involved, all agree that

This investigation was aided by grants to William T. Salter from the International Cancer Research Foundation and from the Committee on Research in Endocrinology of the National Research Council.

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1. Throughout this paper the terms "immunity" and "immunization" are used in a specialized sense to indicate resistance against tumor growth on the part of the host.

2. Woglom, W. H.: The Nature of the Immune Reaction to Transplanted Cancer in the Rat, in Scientific Report on the Investigations of the Imperial Cancer Research Fund, London, 1912, vol. 5, p. 43.

3. Dustin, A. P.: Sang **12**:677, 1938.

4. Allen, E.; Smith, G. M., and Gardner, W. U.: Am. J. Anat. **61**:321, 1937.

5. Brues, A. M., and Cohen, A.: Biochem. J. **30**:1363, 1936.

the results obtained with this drug reflect relative rates of cell multiplication or the "readiness of the cell to divide."[6] For this reason it seemed advisable to compare results obtained (*a*) directly, by determining the percentage of cells in mitosis in untreated tumors, with parallel results obtained (*b*) after preliminary treatment with colchicine.

EXPERIMENTAL PROCEDURE

Pedigreed mice of the Bagg albino strain A were inoculated subcutaneously with sarcoma 180. These animals constituted the control group. Test animals were first "immunized" by the procedure of Andervont[7] or by injections of nonviable tumor extract.[8] The "immunized" animals were then inoculated subcutaneously in the groin with tissue the same as that used for the control animals. Consequently there resulted in the "immunized" mice a graded series of tumors, so that these animals could be classified as having (*a*) no tumors, (*b*) small tumors and (*c*) larger tumors. Indeed, a few of the tumors were very large. On the contrary, all control animals usually had large tumors.

At various intervals of time, ranging from fourteen to forty-five days after inoculation in the groin, test and control animals were put to death simultaneously. The tumors were rapidly removed and fixed in Zenker's solution. Paraffin sections of these tumors were prepared and stained with iron hematoxylin and eosin. The tumors from the control animals are hereafter referred to as the controls, and the tumors from the "immunized" mice, as the "immunes." The mitoses were enumerated by the method previously described by Brues and Marble.[9] Each result was recorded as the percentage of tumor cells undergoing mitosis in groups of at least 1,000 counted cells. All stages of mitosis were included, from the disappearance of the nuclear membrane in prophase to the reappearance of normal nuclear configuration in telephase.

Colchicine.—Similar observations were made in parallel experiments with colchicine. In preliminary tests a few mice were given varying doses of the alkaloid subcutaneously for the purpose of titrating the effect of the drug on mitosis by bioassay. In this experiment the percentage of cells in each of the various stages of mitosis was determined and also the percentage of those in abnormal stages, i. e., lacking spindles. The results of this preliminary titration of the effect of colchicine are shown in table 1. They indicate that a dose of 0.025 mg. in a 25 Gm. mouse gave optimal exaggeration of mitotic activity. Incidentally, this increase in mitoses was made up largely of abnormal forms. Accordingly, 25 micrograms of colchicine was given subcutaneously to parallel series of control and "immune" adult mice from eight and one-half to nine hours prior to killing the animals.

EXPERIMENTAL RESULTS

A comparison of the number of mitoses in control and in "immunized" tumors, both with and without preliminary colchicine treatment, is shown in table 2. These data constitute a complete experiment, designated as group A. It can be seen that without colchicine mitoses are most numerous in controls during the first thirty days after inocula-

6. Dustin, A. P.: Compt. rend. Assoc. d. anat., Basel, April 10-14, 1938.
7. Andervont, H. B.: Pub. Health Rep. **47**:1859, 1932; **49**:60, 1934.
8. Salter, W. T., and Wilson, H.: Unpublished data.
9. Brues, A. M., and Marble, B. B.: J. Exper. Med. **65**:15, 1937.

TABLE 1.—*Percentage and Types of Mitotic Figures*

				Percentage of Tumor Cells in Given Stage of Mitosis					
	Colchicine Dose, Mg.			Pro-phase	Meta-phase†	Ana-phase	Tele-phase	Abnormal Figures	All Stages
Titration of colchicine effect *	0			0.15	0.50	0.35	0.05	....	1.05
	0.010			0.20	1.00	0.30	0	....	1.50
	0.025			0.10	1.20	0.30	0.10	4.50	6.20
	0.060			....	....	....	....	1.10	....
Distribution of mitoses by phases in tumors in group A	Controls treated with colchicine			0.2	0.2	0.15	0.15	1.5	
				0	0.7	0.2	0.2	1.1	
				0	0.4	0.4	0.2	5.7	
				0	0.1	0.1	0.2	3.6	
				0.1	0.8	0	0.1	13.5	
				0	0.7	0.2	0.1	7.8	
				0.1	0.5	0.2	0.1	4.3	
	Mean values			0.06	0.49	0.18	0.15	5.90	
	Mean values for 10 tumors not treated with colchicine			0.02	1.38	0.31	0.07	0.02	
	Immunes treated with colchicine			0.05	0.05	0.05	0.05	0.65	
				0	0.6	0	0.1	2.4	
				0	0.8	0.4	0	5.3	
				0	1.1	0.2	0.1	1.9	
				0	0.2	0.1	0	0.8	
				0	0.2	0.2	0	1.7	
				0	0.1	0.1	0.1	0.5	
				0	0.1	0.1	0	1.5	
				0.1	0.3	0.1	0.2	4.4	
				0.1	0	0	0	5.1	
				0	0.4	0	0	2.7	
				0	0.4	0.6	0.2	5.8	
				0	0	0.1	0.1	1.2	
				0.1	0.4	0.1	0.2	4.3	
	Mean values			0.02	0.25	0.13	0.07	2.45	
Mean percentage of cells in mitosis by phases in tumors of group B		Time After Inoculation, Days	No. in Series						
	Immunes	25	16	0.003	0.63	0.22	0.04	0.03	0.92
	Controls	25	29	0.03	0.98	0.42	0.06	0.02	1.46
	Controls	35	5	0.01	0.72	0.17	0.09	0.03	1.02

* C57 mice bearing sarcoma 180 were used in this experiment. Colchicine was given subcutaneously nine hours before the mice were killed.

† The figures refer to cells in normal metaphase.

TABLE 2.—*Statistical Analysis of Tumor Size and Mitotic Activity*

Group A. Percentage of cells in mitosis in controls and immunes with and without colchicine treatment	Tumors Treated with Colchicine		Tumors Not Treated with Colchicine			
	Controls: 15-32 Day Tumors	Immunes: 15-28 Day Tumors	15-29 Day Tumors	30-46 Day Tumors	13-25 Day Tumors	31-45 Day Tumors
	6.9	5.6	2.7	1.6	0.7	0.4
	4.0	3.9	2.8	1.4	2.3	1.2
	6.2	6.2	0.8	1.0	1.7	1.5
	12.8	3.2	1.2	2.1	1.4	1.4
	8.9	2.8	1.8	0.9	2.3	1.7
	10.0	0.9	3.3	1.5	2.4	2.7
	4.0	3.1	2.1	1.9	1.7	0.6
	2.2	6.5	2.7	0.8	1.2	2.2
	1.1*	13.3	...	0.8	1.4	2.5
	6.6	2.3	...	1.4	1.9	0.8
	4.0	1.1	...	1.0	...	1.9
	4.1	2.0	...	2.1	...	...
	14.5	0.8	...	0.9	...	...
	8.8	1.9	...	1.5	...	...
	5.2	5.1	...	1.9	...	...
	...	5.2	...	...	...	...
	...	3.1	...	...	...	...
	...	1.0	...	...	...	...
	...	3.7	...	...	...	...
	...	7.0	...	...	...	...
	...	1.4	...	...	...	...
	...	5.1	...	...	...	...
Mean value	6.62	3.87	2.17	1.37	1.71	1.54
Standard deviation	3.53	0.60	0.83	0.45	0.54	0.73

	Percentage of Cells in Mitosis		Length of Tumor, Mm.	
Group B. Mitotic activity and tumor size	Controls	Immunes	Controls	Immunes
Median	1.25	0.87	21.0	11.0
Mean	1.46	0.92	21.0	12.3
Standard deviation	0.53	0.47	5.6	5.4
Coefficient of variation	0.36	0.51	0.37	0.44

* This was a thirty-two day tumor.

tion and that their number is decreased slightly in "immunes" at all intermediate intervals of time within this period. The older controls (i. e., after thirty days) also have a low rate of mitosis.

After preliminary treatment with colchicine the same general relationships hold between controls and "immunes" as in the untreated series. The variability in all groups was rather large, as indicated by the standard deviations. This variability seemed due in large part to variability in the sizes of the respective tumors. There was striking consistency in the distribution of mitotic phases in the two groups, as seen in table 1, group A. In general, the averages of each phase in the control group showed twice the number of the "immune" group. Even more noticeable was the parallelism between mitosis and tumor

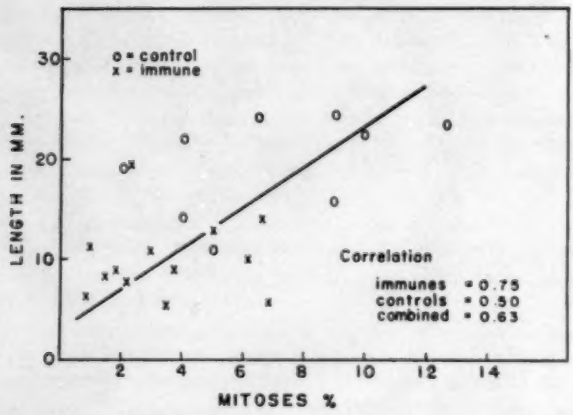

Fig. 1.—Relation between tumor size (expressed as length) and percentage of cells in mitosis ten hours after colchicine treatment and eighteen days after inoculation of mice with tumor. The coefficient of correlation for controls and "immunes" combined equals $+0.63 \pm 0.09$.

size. The values for a characteristic experiment with colchicine, in which all animals were put to death at the same time (eighteen days after inoculation with tumor), are shown in figure 1. It can be seen that there is good correlation between mitosis (after treatment with colchicine) and tumor diameter. It will be observed also that a single tumor in the "immune" group gave aberrant values for both size and mitoses; in short, "immunity" had failed to develop in this animal, possibly through lapse of technic. The coefficient of correlation between size of tumor and rate of mitosis for the mixed data (controls and "immunes" combined) is plus 0.63; for the "immunes" alone, plus 0.75; for the controls alone, plus 0.50.

In the tumors given preliminary colchicine treatment there is a great increase in the total mitosis count over that found in the untreated. This increase is obviously due to the presence of large numbers of abnormal figures, which may be classed as pseudometaphases in which the mitotic spindle is lacking. There are many dividing cells in which the spindle is present, however, and the late stages of mitosis are found in virtually all of these tumors. These cells, which show evidence of normal karyokinetic activity, are approximately half as numerous as in the tumors not treated with colchicine. This bears out an earlier observation on sarcoma 180 [10] and suggests that in this tissue the abnormal figures represent only a prolongation of cell division.

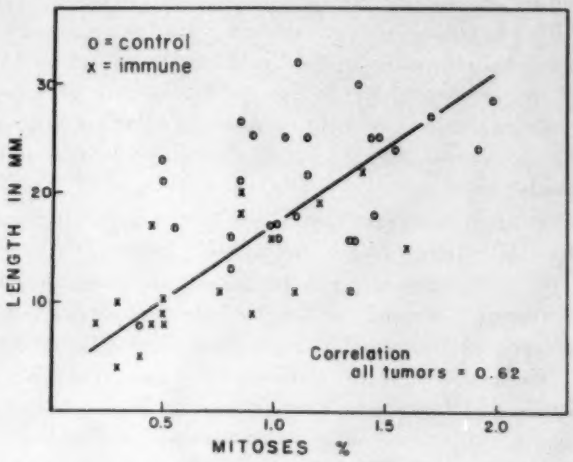

Fig. 2.—Relation between tumor size and percentage of cells in mitosis in tumors from mice not treated with colchicine twenty-five days after inoculation. The coefficient of correlation for controls and "immunes" combined equals $+0.62 \pm 0.06$.

A separated series of tumors, group B, was studied without colchicine treatment, as shown in table 1. For convenience, the data for "immunes" and controls are summarized in table 2, group B and in figure 2. Here, again, there was good correlation between size of tumor and rate of mitosis, i. e., 0.62 for the entire group of "immunes" and controls, combined. There was likewise good correlation in the distribution of mitotic phases in the two groups, as seen in table 2. The ratio between homologous values was only about 1.6, instead of 2.0, as in group A. Nevertheless, the same striking parallelism existed between mitosis and size in the two groups, as demonstrated by the

10. Brues, A. M., and Jackson, E. B.: Am. J. Cancer **30**:504, 1937.

data shown. The ratio between mean values for controls and "immunes" is 1.7 with respect to length of tumor and 1.6 with respect to rate of mitosis.

COMMENT

These data show that during the period of active growth of tumors inoculated in mice, whether treated with colchicine or not, the more rapidly growing tissues exhibit a higher percentage of cells in mitosis. Consequently, the inhibition of growth produced by "immunization" actually does involve inhibition of the rate of cell division. Indeed, it appears that the "immune" and control tumors form a statistical continuum from small tumors with few mitoses to large tumors in which mitotic activity is great.

After a time the tumors inoculated in control animals assume the mitotic activity of young tumors growing in "immunized" animals. This phenomenon corresponds to the "concave growth curve" described by Schrek [11] and confirmed by Salter and Wilson.[8] This retardation presumably reflects the development of resistance to neoplasm occurring spontaneously in control animals, even though it is not sufficient to save the animals' lives.

The present figures suggest that about thirty days after inoculation with sarcoma 180 spontaneous "immunity" appears in the control animals, for the percentage of cells in mitosis drops to approximately that of the "immune" group. Although there is great variation in the percentage of cells in mitosis under all these various combinations of experimental variables, it seems considerably less than that recorded by Brues, Marble and Jackson [12] for regenerating liver. For this reason, it appears that a simple enumeration of mitoses in histologic sections of this tumor *without* preliminary colchicine treatment constitutes a good measure of the rate of cell multiplication. The colchicine technic is of greater value in the case of tissues in which the rate of mitosis is very low or fluctuates from hour to hour.

SUMMARY

A series of "immune" tumors (sarcoma 180 in strain A mice) shows a significantly lower mean mitotic rate than is seen in their controls. The mitotic rate is closely correlated with size in individual tumors, and in these characteristics immunized tumors and controls are shown to form a continuous series.

The effect of colchicine is to exaggerate the visible evidence of mitotic division. This exaggeration, as might be expected, is propor-

11. Schrek, R.: Am. J. Path. **12**:525, 1936.

12. Brues, A. M.; Marble, B. B., and Jackson, E. B.: Am. J. Cancer **38**:159, 1940.

tionally higher in the group having a higher rate. It does not appear that the colchicine technic is necessary in the estimation of rates of mitosis in tumors.

The process of "immunity" to tumors involves retardation of the mitotic rate. This mitotic criterion of immunity appears in control tumors about thirty days after the mice have been inoculated, suggesting self immunity.

ALVEOLAR LINING OF THE LUNG IN RELATION TO THE VIABILITY OF THE FETUS

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The presence or absence of a layer of epithelium lining the walls of the pulmonary alveoli has been the source of considerable controversy. For over eighty years, since the subject was first introduced by Thomas Addison, such authorities as Chrzonszczowsky, Kölliker,[1] Eberth, Elenz, Colberg and, more recently, Lang, Rose,[2] Maximow and Bloom,[3] Clara,[4] Barnard and Day,[5] Miller[6] and Cooper[7] have reported conflicting results from histologic investigation of the alveolar lining. The controversy has narrowed down to the question of whether there is a continuous layer of epithelium, one made up of large non-nucleated plates interspersed with islands of small nucleated cells, or no alveolar cell lining at all but merely the walls of the capillaries surrounding the alveolar spaces. Miller[6] stated that the great obstacle to a correct understanding of the alveolar epithelium was the inability to dissect off the epithelial layer or to remove it by artificial means. He was able to overcome this difficulty by observing pathologic sections (e. g., in cases of pneumonia) in which the epithelium was pushed off by the pouring of serous exudate behind it. Barnard and Day[5] investigated 48 human fetal lungs and came to the conclusion that although the early alveolar spaces were lined by pseudostratified epithelium, this lining began to disappear after the sixth month of embryonic life and did not reappear. Cooper[1] demonstrated by histologic sections that

From the Department of Pathology of the University of Illinois College of Medicine, the Department of Pathology of Cook County Hospital and the Children's Division of Cook County Hospital.

1. Kölliker, A.: Verhandl. d. phys.-med. Gesellsch. in Würzburg **16**:1-24, 1881.
2. Rose, S. B.: Arch. Path. **6**:36, 1928.
3. Maximow, A., and Bloom, W.: Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1934.
4. Clara, M.: Ztschr. f. mikr.-anat. Forsch. **40**:147, 1936.
5. Barnard, W. G., and Day, T. D.: J. Path. & Bact. **45**:67, 1937.
6. Miller, W. S., in Cowdry, E. V.: Special Cytology, ed. 2, New York, Paul B. Hoeber, Inc., 1932; The Lung, Springfield, Ill., Charles C. Thomas, Publisher, 1937.
7. Cooper, E. R. A.: J. Path. & Bact. **47**:105, 1938.

there was a continuous alveolar epithelium which became somewhat attenuated in later fetal life.

Although the literature has presented voluminous discussions of the histologic nature of the alveolar lining, little attention has been given to the relationship between the type of cells lining the alveoli and the viability of the fetus. It is agreed that, whether the origin is ectodermal or mesodermal, large cuboidal cells make up the lining of the alveoli of young embryos and that after five or six months of intrauterine life these cells become flattened. The exact details of the supposed metamorphosis of the cuboidal "epithelium" into flat plates interspersed with small inconspicuous cells of the alveolar walls have not been thoroughly described. Some have claimed that this transformation occurs in the later embryonic stages by distintegration of the nuclei, degeneration of the cytoplasm and casting off of the cells. Others have stated that the change is accomplished by an encroachment of capillaries and desquamation of the "epithelium."

Whatever the origin of the cells and the nature of their transformation, the following questions arise: Are these large cuboidal cells compatible with life? Do they persist after five or six months of embryonic life as a pathologic phase of an earlier physiologic process? What relationship have they to livebirth and stillbirth of infants? Does the transition from nonrespiratory functioning cuboidal cells to respiratory functioning flat cells coincide with the transition from a previable to a viable infant?

The cause of stillbirths and neonatal deaths with no definite pathologic evidence of disease or injury has puzzled obstetricians, pediatricians and pathologists for many years, particularly when infants of viable age were born of healthy parents and by normal, uncomplicated deliveries. Potter,[8] in a recent survey of a large number of fetal deaths, showed that in only 57 per cent of the cases could a definite pathologic state be determined at autopsy. Public health statistics substantiate this observation—the difficulty encountered in establishing the cause of death during the neonatal period. In many instances the autopsy revealed only pulmonary atelectasis, which is not regarded as a primary cause of death but is interpreted as a secondary factor. Prematurity alone could not always be the cause of death, because some infants weighing 1,000 Gm. survived, while many of those at term did not. Histologically, if one could demonstrate persistence of immature alveolar cells in a viable infant, the cause of death might be partially clarified.

Because of these problems, it was decided to study the lungs of human fetuses to determine the chronologic intrauterine age at which

8. Potter, E. L.: J. A. M. A. **112**:1549, 1939.

the transition from cuboidal to flat cells occurs and to determine the relation between maturity of the alveolar cell lining and viability of the fetus.

MATERIAL

The lungs of 50 newborn infants were examined grossly and microscopically. These lungs were obtained from liveborn and stillborn infants. The infants were subdivided, according to Scammon's classification (based on weight in grams, crown-heel length in centimeters and estimated intrauterine age), into abortive, previable premature, viable premature, full term and postmature groups.

METHODS

Clinical Study.—In order to establish a complete clinical background for each specimen studied histologically, data on the following points were recorded for each infant: weight, height, sex, color, mother's last menstrual period, expected date of delivery, actual date of delivery, estimated intrauterine age, condition of infant at birth, signs of maturity or immaturity at birth, age of infant at death, mother's serologic record, and hours the infant was dead before autopsy was performed.

Gross Pathologic Examination.—The weight, the crown-heel and crown-rump height, and the external evidence of maturity were noted. The position of the lungs in the thoracic cavity was observed. The color and consistency of the lungs were recorded. The lungs were tested to see if they would float in water; the individual lobes were also tested in this manner. The cut section was studied. The trachea and bronchi were examined for patency or occlusion by mucus.

Fixation and Staining of Tissues.—Four pieces of tissue were fixed in each case studied; these included a section from (*a*) the apex of the right lung; (*b*) the hilus of the upper lobe of the left lung; (*c*) the hilus of the lower lobe of the right lung, and (*d*) the base of the lower lobe of the left lung. The tissue from the right lung was fixed in Schaffer's [8a] fluid, and that from the left lung was fixed in Carnoi solution.[8a] After fixation, the tissues were embedded and blocked in the usual manner.

The sections were stained routinely with hematoxylin and eosin. In some cases, additional sections were treated with elastin H, azan and fibrin stains.

Microscopic Examination.—The state of expansion or collapse of the alveoli was noted. The cells lining the alveolar spaces were studied in great detail as to whether they were cuboidal or flattened or showed a combination of these types. The contents of the alveoli and bronchi were noted. Attention was also given to any unusual features, such as atypical cells or evidence of aspirated material.

RESULTS

Age and Classification of Infants.—The lungs of 50 newborn infants were studied. Of these infants, 20 were born alive and 30 were stillborn.

8a. The composition of the fixing fluids is as follows: (*a*) Schaffer's fluid—2 parts of 80 per cent alcohol and 1 part of 40 per cent solution of formaldehyde; (*b*) Carnoi solution—6 parts of absolute alcohol, 3 parts of chloroform and 1 part of glacial acetic acid.

The duration of life in the liveborn was from a few minutes to forty-five days. With the exception of 2 infants (one living forty-five days and the other fifteen days) none lived for more than twenty-seven hours.

The intrauterine age as estimated from the time of the last menstrual period varied from 12.4 weeks to 44.7 weeks. According to Scammon's classification, 14 infants were in the abortive group, 12 in the previable premature group, 18 in the viable premature group, 5 in the full term group and 1 in the postmature group.

Type of Alveolar Lining Cells Found.—Large, immature, cuboidal cells were found lining the alveoli of the lungs in 20 cases (figs. 1 and 2). In 15 cases there was a mixture of cuboidal and flat, mature cells lining

Relation Between Intrauterine Age and Type of Cells Lining the Alveoli of the Lungs

Intrauterine Age, Weeks	Type of Cells		
	Cuboid	Cuboid and Flat	Flat
12 to 16 (4 cases)	4	..	..
16 to 20 (5 cases)	4	1	..
20 to 24 (10 cases)	8	2	..
24 to 28 (11 cases)	3	7	1
28 to 33 (10 cases)	1	4	5
33 to 37 (4 cases)	..	1	3
37 to 45 (6 cases)	..	..	6

the alveoli (fig. 3). In the remaining 15 cases there was a complete flattened alveolar lining such as that seen in the adult lung.

Type of Cells Compared with Intrauterine Age.—A cuboidal, immature, nonrespiratory functioning type of cell was found lining the alveoli of all the fetuses under 16 weeks of intrauterine age and of 80 per cent of those under 24 weeks of intrauterine age.

A flattened, mature, respiratory functioning type of cell was found lining the alveoli of all infants that were over 37 weeks of gestational age and of 75 per cent of those over 33 weeks of gestational age.

A mixture of cuboidal and flattened cells was noted most frequently between the twenty-fourth and twenty-eighth week and almost as frequently between the twenty-eighth and thirty-third week of intrauterine age (table).

Type of Cells According to Scammon's Classification.—Computations based on estimated intrauterine age are always subject to error, and it was deemed advisable to compare the type of alveolar cells with

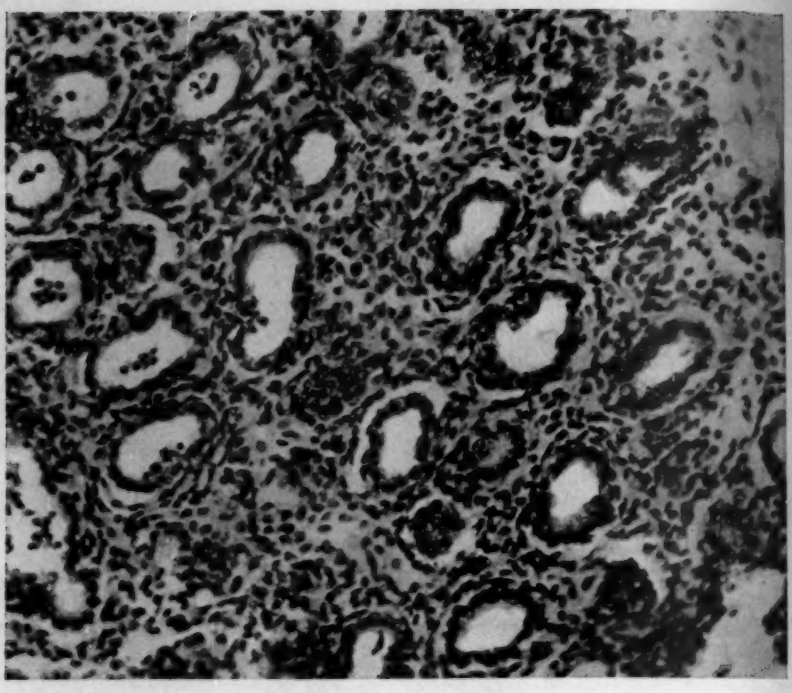

Fig. 1.—Lung of a stillborn infant (weight, 400 Gm.; length, 27 cm.; intrauterine age, 21 weeks) of the abortive group. Cuboidal cells form the lining of all the alveoli.

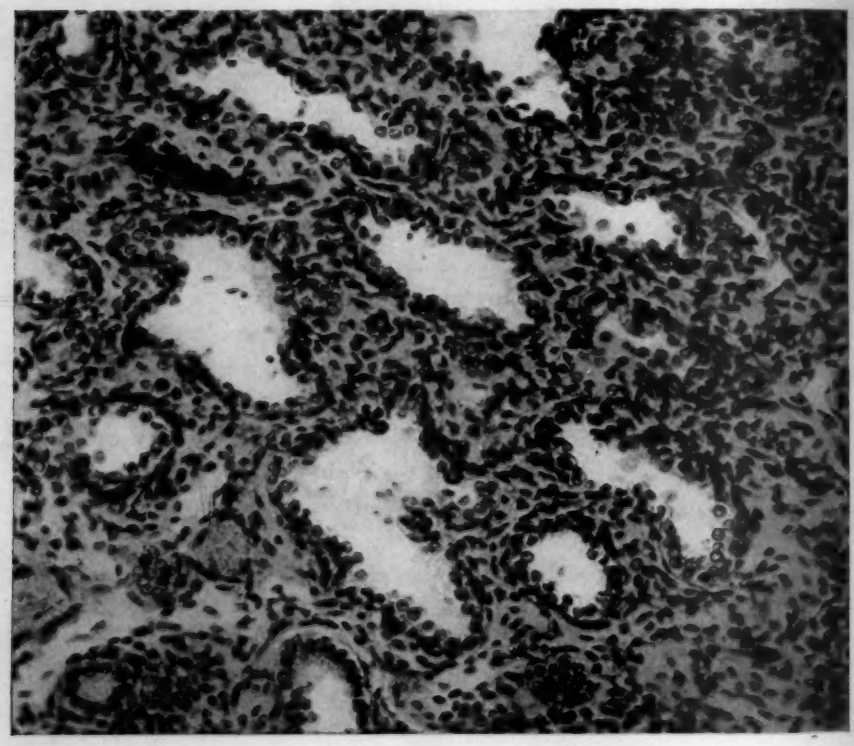

Fig. 2.—Lung of an infant who lived fifty minutes (weight, 550 Gm.; length, 30 cm.; intrauterine age, 22 weeks) of the previable premature group. Cuboidal cells form the lining of all the alveoli.

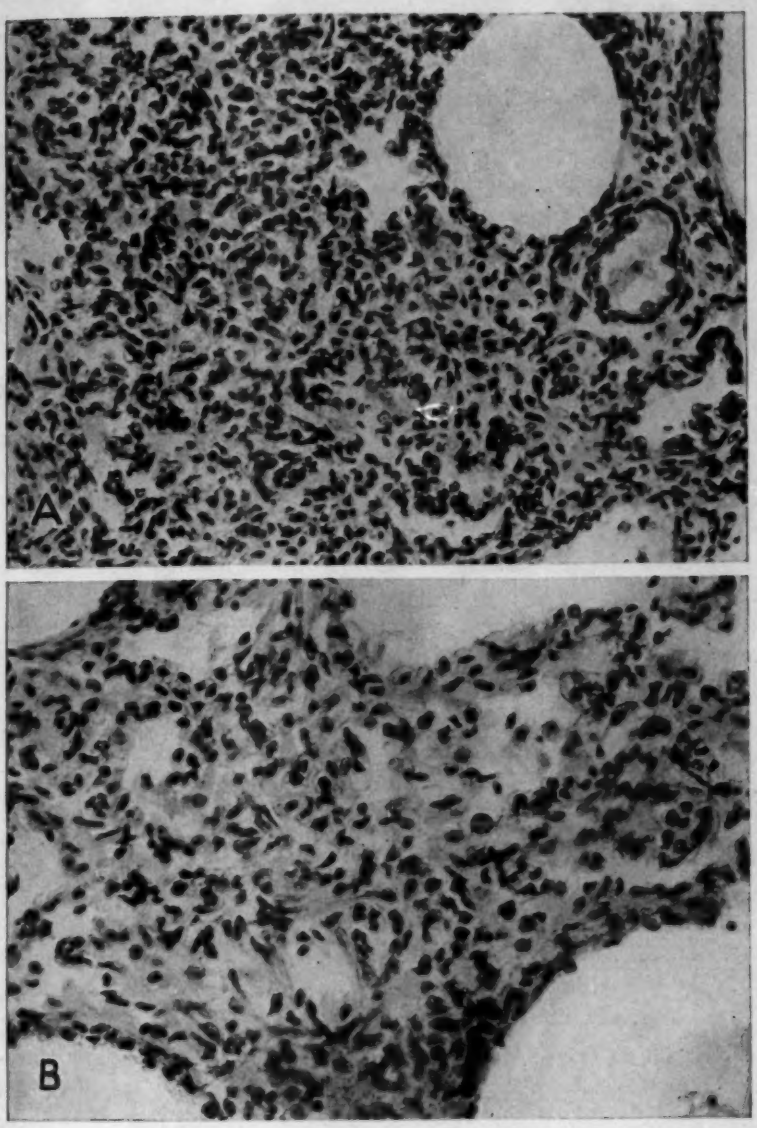

Fig. 3.—*A*, lung of a stillborn infant (weight, 1,225 Gm.; length, 39 cm.; intrauterine age, 21 weeks) of the viable premature group. There is a transition from cuboidal cells lining the alveoli in some areas to flattened cells in other areas. *B*, lung of an infant who lived 45 days (weight, 850 Gm.; length, 38 cm.; intrauterine age, 17.8 weeks) of the previable premature group. There is a transition from cuboidal cells lining the alveoli in some areas to flattened cells in other areas.

the grouping devised by Scammon, instead of relying solely on the computed gestational age. This classification, briefly, is as follows:

1. Abortive Group
   - Length less than 28 cm.
   - Weight less than 400 Gm.
   - Gestation less than 22 weeks.
2. Premature Group
   - (*a*) Previable
     - Length from 28 to to 34.9 cm.
     - Weight from 400 to 999 Gm.
     - Gestation from 22 through 27 weeks.
   - (*b*) Viable
     - Length from 35 to 46.9 cm.
     - Weight from 1,000 to 2,499 Gm.
     - Gestation from 28 through 36 weeks.
3. Full Term Group
   - Length from 47 to 54 cm.
   - Weight from 2,500 to 4,500 Gm.
   - Gestation from 37 to 43 weeks.
4. Postmature Group
   - Length more than 54 cm.
   - Weight more than 4,500 Gm.
   - Gestation more than 43 weeks.

In any case in which the three variables did not fall into one group, the infant was considered as in that group in which two of the three factors were present.

The entire abortive group of 14 fetuses was characterized by a complete cuboidal lining of the alveoli. Of the 12 infants in the previable premature group, half presented an immature cuboidal lining, while the remaining ones revealed a combination of cuboidal and flat cells. In the viable premature group 9 infants were noted to have a mixed lining, and the other 9 revealed a complete mature, flattened cell layer. In 4 of the 5 infants in the full term group a mature, flattened "epithelium" was observed, while in 1 infant there was a mixture of cuboidal and flat cells. The single postmature infant revealed a complete flattened lining, such as is seen in the adult lung.

Type of Cells in Relation to Liveborn and Stillborn Infants.—Twenty of the infants studied were born alive. Thirty were stillborn.

An investigation of the cases of livebirth revealed that the alveolar lining cells were flat and mature in only 7 cases. Four of the infants who were born alive and lived up to three and one-half hours had a complete layer of immature, cuboidal cells lining the alveoli. Nine infants had a transitional type of cell lining, composed of both cuboidal and flat cells.

The clinical history of the 4 infants who were born alive and were later noted to have an immature alveolar cell lining incompatible with respiration revealed that the duration of life was from a few minutes to three and one-half hours. The weights varied from 265 to 980 Gm. Two of these infants belonged to the abortive group. Two belonged to the previable premature group. All 4 infants were delivered in spontaneous abortion; 2 in breech presentation. The postmortem examination showed prematurity and atelectasis in 3 of these infants and, in addition, subdural and subtentorial hemorrhage in 1.

Among the 9 infants presenting a mixed lining of both cuboidal and flattened cells were the infants who lived forty-five and fifteen days, respectively. Their deliveries were spontaneous and normal. The infant who lived forty-five days weighed 850 Gm. at birth and belonged to the previable premature group. At autopsy, severe icterus and serous hepatitis were present. The infant who lived fifteen days weighed 1,395 Gm. and belonged to the viable premature group. The postmortem examination revealed anomalies of the bladder, intersexuality and absence of the abdominal muscles.

The duration of life of the remaining infants of this group was from twenty minutes to six hours. The weights of these infants varied from 750 to 1,750 Gm. Two of the infants belonged to the previable premature group. Five belonged to the viable premature group. The deliveries of the entire group were spontaneous and normal. Two of the subjects were twins, and 1 was born in breech presentation; there was associated polyhydramnios, but the delivery was short and uncomplicated. There were 2 others who were in breech presentation in this group, and 1 who was in compound presentation. Since the alveolar "epithelium" composed of cuboidal and flat cells in this group had the potentiality of respiration, the possible causes of death were carefully investigated. In addition to the previously mentioned infants showing, at autopsy, anomalies and serous hepatitis, 4 revealed only atelectasis and prematurity, and, of the others, 1 infant presented a subtentorial hemorrhage and 1 a subcapsular hemorrhage of the liver.

The duration of life in the 7 infants whose alveolar lining was completely mature and capable of respiration was from one hour and twenty-five minutes to twenty-seven hours. The weights varied between 910 and 2,915 Gm. Four of these infants were premature but viable, 1 was previable and premature, 1 was full term, and 1 was postmature. Three of the infants were born by easy spontaneous deliveries. Two were born by cesarian section; one section was done in a case of hypertension and the other in a case of placenta praevia. One infant was in breech presentation at birth and was delivered with forceps on the after-coming head. The last case was one of placenta praevia in which

the infant was delivered by version and extraction. At autopsy 5 infants revealed only prematurity and atelectasis; 1, in addition, presented slight hypertrophy of the heart. The other 2 infants showed pathologic changes as follows: one, an achondroplastic dwarf, subdural hemorrhage and macrocephaly; the other, bilateral hydroureter, distention of the urinary bladder and congestion of the brain.

Of the 30 stillborn infants, 16 presented immature cuboidal "epithelium" incompatible with respiratory function, 6 had both cuboidal and flat cells, and 8 revealed a mature flattened lining.

Investigation of the 8 infants who were stillborn and had a mature alveolar lining capable of function revealed that 3 were full term and 5 were premature but viable. Six of these infants were delivered in a normal manner. One, in a case of placenta praevia, was delivered by cesarian section, and another was delivered by postmortem section on a mother who died of tuberculous meningitis. At autopsy the infant in the case of placenta praevia revealed subcapsular hemorrhages of the liver and lungs, indicating asphyxia. The mother of an infant in whom only atelectasis was present died six hours post partum with a diagnosis of sickle cell anemia. One infant died from cerebral hemorrhage. The other infants of this group revealed only atelectasis at death.

COMMENT

The study of the lungs of 50 newborn infants revealed that a complete cuboidal alveolar lining is incompatible with life. This type of lining was exclusively present in every infant in the abortive group and in 50 per cent of those in the previable premature group.

The persistence of life in the previable premature group—for example, in this investigation, 1 previable infant weighing only 850 Gm. lived forty-five days—may be accounted for by a partially mature alveolar lining. In the previable group, although half of the group presented a completely immature lining, the other half of the group revealed a mixture of cuboidal cells and mature flattened cells.

In the viable premature group, however, there was persistence of some immature, cuboidal alveolar cells, which might account for the death of an otherwise normal infant. Only half of the infants in the viable group revealed a completely mature alveolar lining; the others showed a mixed layer of cuboidal and flat cells. It was interesting that in this viable group there was not one infant that presented a complete immature, cuboidal lining; in other words, the potentiality for the function of respiration was always present.

In the full term group, in which viability is expected, 4 of the 5 infants revealed a fully matured cell lining; 1 infant, however, presented a mixed lining in which cuboidal cells still persisted.

This investigation also showed that the period of transition from cuboidal to flattened "epithelium" occurred in the greatest percentage of the cases between the twenty-fourth and thirty-third week of gestational age, or at the time that the previable infant becomes viable. Over 60 per cent of the infants between 24 and 28 weeks and 40 per cent between 28 and 33 weeks of intrauterine age presented this mixed type of alveolar lining.

Among the liveborn infants it was noted that the longest duration of life in those whose alveoli were composed entirely of immature, cuboidal cells was three and one-half hours. The duration of life in those infants whose lungs were lined by a mixture of cuboidal and flattened cells was forty-five days and fifteen days in 2 and up to fourteen hours in the others. Furthermore, of the 20 infants who were born alive, 12 gave no evidence of disease, anomaly or injury at autopsy. In these 12 cases of "unexplained death" the duration of life was from a few minutes to twenty-one hours. Two of the subjects were in the abortive, 2 in the previable premature and 8 in the viable premature group. In 3 of the 12 cases there was a complete immature cell lining, incompatible with life; in 4 a mixture of cuboidal and flattened cells and in 5, a mature alveolar lining which was capable of respiratory function. Therefore, 7 of the 12 "unexplained deaths" could be accounted for by either a completely immature or partially immature alveolar cell lining; the remaining 5 could not be explained.

In comparing the infants who were born alive with those who were stillborn, it was found that only 4 of the 20 liveborn infants as compared with half of the 30 stillborn ones had a complete immature cuboidal lining. It was also noted that 35 per cent of the liveborn infants as compared with 20 per cent of the stillborn ones presented a mature flat alveolar lining. In other words, a potential respiratory functioning, lining was noted more frequently in the infants who were born alive than in those who were stillborn.

SUMMARY

The entire abortive group and the greatest percentage of the previable premature group revealed an immature, cuboidal alveolar lining, which is not compatible with respiration. The greatest percentage of the full term group revealed a flattened, mature cell lining such as that seen in the adult lung.

The transition from cuboidal immature to flattened mature alveolar lining cells was noted most frequently between the twenty-fourth and thirty-third week of intrauterine life, or at the time that the previable premature infant becomes viable.

Cuboid, nonrespiratory functioning cells persisted in 50 per cent of the viable premature infants and in 20 per cent of the full term infants. This persistence of immature cells in viable infants as a pathologic phase of an earlier physiologic process is significant in the deaths of otherwise normal infants. In a study of 12 unexplained deaths in which no pathologic condition could be discerned at postmortem examination, it was observed that 7 of the infants had a complete or partial immature alveolar cell lining.

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GOLGI APPARATUS OF THE THYROID GLAND

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A reticular material called the Golgi apparatus is present in all differentiated cells. It can be demonstrated under the microscope by special fixation of the tissue and by impregnation with silver. This reticular material is particularly well developed in the cells of the nervous system and in the secretory cells of glands. Study of the Golgi apparatus of the thyroid gland under various biologic conditions has yielded some definite information as to the relation between the size of the apparatus and the physiologic activity of the cell.

Hirschlerowa [1] observed hypertrophy of the Golgi apparatus in the thyroid cells of amphibian larvae undergoing metamorphosis. She later found an enlarged Golgi apparatus in the thyroid gland of a patient with exophthalmic goiter. Further studies by Ludford and Cramer [2] demonstrated that exposure to cold brought about hypertrophy of this reticular material in the thyroid glands of rats and mice. It seems to be generally agreed that enlargement of the Golgi apparatus in the cells of glands is evidence of increased secretory activity.

Other investigators have attempted to attach some significance to the position of the Golgi apparatus within the cell. Its usual position is between the nucleus and the secretory surface. Cowdry [3] suggested that in the case of the thyroid gland a basal position of the apparatus might be an indication of a secretion of toxic products directly into the blood stream. Other workers [4] could not ascribe any significance to the position of the apparatus, and on the whole this line of investigation proved unfruitful. There is, however, no doubt of the value of an estimation of the size of the Golgi apparatus of the thyroid gland,

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1. Hirschlerowa, Z.: Ztschr. f. Zellforsch. u. mikr. Anat. **6**:234, 1927.

2. Cramer, W., and Ludford, R. J.: J. Physiol. **61**:398, 1926. Ludford, R. J., and Cramer, W.: Proc. Roy. Soc., London, s.B **104**:28, 1928.

3. Cowdry, E. V.: Am. J. Anat. **30**:25, 1922.

4. (*a*) Okkels, H.: Acta path. et microbiol. Scandinav. **9**:1, 1932. (*b*) Gilman, J.: South African J. M. Sc. **1**:97, 1935. (*c*) Hirschlerowa.[1]

as Okkels and Krogh and Lindberg [5] showed in their study of goiters. In a series of papers they called attention to the constancy of the finding of an enlarged Golgi apparatus in the cells of thyroid glands removed from patients with toxic goiters. Included were some thyroid glands from patients with exophthalmic goiter and some from patients with adenomatous goiter with hyperthyroidism. The size of the Golgi apparatus did not seem to be affected by the preoperative administration of iodine, although the glands usually showed regression to a state approaching that of the colloid thyroid in appearance.

The Golgi apparatus of thyroid gland cells has been studied in various types of goiter, and the classification referred to in a previous article by one of us (Broders [6]) has been used to differentiate abnormal thyroid conditions. This classification, which evolved from the clinical and pathologic conceptions of the thyroid gland of Wilson,[7] MacCarty,[8] H. S. Plummer,[9] Boothby [10] and Broders,[6] importantly differentiates adenomatous goiter from exophthalmic goiter.

MATERIAL AND METHOD

The Golgi apparatus was examined in 73 thyroid glands removed at operation. DaFano's [11] technic was used to demonstrate it. Portions of glands were taken from representative areas, part of which were prepared by the DaFano method. Fresh sections were stained by Terry's [11a] neutral polychrome methylene blue, and sections fixed in 4 per cent solution of formaldehyde, by hematoxylin and eosin; the sections had been cut by the freezing method. Estimations of the size of the Golgi apparatus were made without previous knowledge of the clinical diagnoses. The diagnosis of cellular hypertrophy in these cases was made from frozen sections by members of the staff of the Section on Surgical Pathology. The size of the Golgi apparatus was designated either as "enlarged" or "not enlarged," no attempt being made to differentiate various degrees of enlargement. In doubtful cases the classification was "not enlarged." Table 1 summarizes the pathologic observations.

HISTOLOGIC AND CYTOLOGIC OBSERVATIONS

Parenchymal Cellular Hypertrophy of the Thyroid Gland.—The gross and microscopic characteristics of the thyroid gland which are associated with various types of thyroid disease are well known. We have

5. Krogh, M., and Lindberg, A. L.: Acta path. et microbiol. Scandinav. **9**:21, 1932. Krogh, M.; Lindberg, A. L., and Okkels, H.: ibid. **9**:37, 1932. Krogh, M., and Okkels, H.: Compt. rend. Soc. de biol. **112**:1694, 1933. Okkels, H., and Krogh, M.: Acta path. et microbiol. Scandinav. **10**:118, 1933. Okkels, H.: Compt. rend. Soc. de biol. **112**:1691, 1933; footnote 4a.

6. Broders, A. C.: Texas State J. Med. **31**:608, 1936.

7. Wilson, L. B.: Am. J. M. Sc. **136**:851, 1908; **147**:344, 1914; **165**:738, 1923.

8. MacCarty, W. C.: New York State J. Med. **12**:595, 1912.

9. Plummer, H. S.: J. A. M. A. **61**:650, 1913; Am. J. M. Sc. **146**:790, 1913.

10. Boothby, W. M.: Endocrinology **5**:1, 1921; J. A. M. A. **74**:1600, 1920.

11. DaFano, C.: J. Roy. Micr. Soc., 1920, pt. 2, p. 157.

11a. Terry, B. T.: J. Lab. & Clin. Med. **14**:519, 1929.

based the histologic descriptions which are briefly presented in this paper on a previous summary.[6] The pathologic diagnosis of parenchymal cellular hypertrophy of the thyroid is regularly made in cases in which the gland is removed from a patient with exophthalmic goiter. The usual microscopic picture of parenchymal cellular hypertrophy of the thyroid is one of follicles lined with high cuboidal or columnar epithelium. There is papillary infolding of the epithelium, and colloid is less abundant. The administration of compound solution of iodine, however, alters the pathologic picture, and at the time of resection a much less severe process than originally was present is seen so far as the hypertrophic changes are concerned.

Now that iodine, as advocated by H. S. Plummer,[12] has come into almost universal use in the preoperative period, one sees less frequently

TABLE 1.—*Microscopic Observations on the Golgi Apparatus in Seventy-Three Thyroid Glands*

Histologic Diagnosis	Total Cases	Golgi Apparatus	
		Enlarged	Not Enlarged
Parenchymal cellular hypertrophy of the thyroid	32	31	1
Single or multiple colloid and fetal adenomas (with or without intra-adenomatous hypertrophy and also with various types of degeneration) in a thyroid with parenchymal hypertrophy	3	3	0
Single or multiple colloid and fetal adenomas (with various types of degeneration) in a colloid thyroid with intra-adenomatous parenchymal hypertrophy	8	7	1
Single or multiple colloid and fetal adenomas (with various types of degeneration) in a colloid thyroid	29	17	12
Colloid thyroid	1	..	1

that marked cellular hypertrophy which was so well known to older pathologists. As Giordano [13] and Rienhoff [14] showed, iodine produces cytologic regression of the gland. The epithelium becomes flattened, colloid accumulates in the follicles, and the gland to a large extent assumes the appearance of a colloid goiter. There are varying degrees of this return of the gland to a more normal state under iodine therapy, but in some instances after prolonged administration of iodine the microscopic picture simulates almost completely that of the colloid thyroid. Although great cellular hypertrophy cannot be seen, there are certain characteristics of this gland, such as lymphocytic infiltration of the interfollicular tissue, the presence of germ centers and the general arrangement of the acini, by which the pathologist may recognize that exoph-

12. Plummer, H. S.: J. A. M. A. **80**:955, 1923.
13. Giordano, A. S.: Arch. Path. **1**:881, 1926.
14. Rienhoff, W. F., Jr.: Arch. Surg. **13**:391, 1926.

thalmic goiter has been present. In exophthalmic goiter the gland usually does not contain adenomas; in less than a fourth of the cases, however, fetal adenomas or adenomatous tissue may be present in a thyroid gland exhibiting diffuse parenchymal hypertrophy. In some cases of adenomatous goiter, also, parenchymal hypertrophy may occur with or without intra-adenomatous hypertrophy. This condition is pathologically different from other types of adenomatous thyroid disease and clinically may be distinguished as exophthalmic goiter, as we shall point out later.

Fig. 1.—Parenchymal cellular hypertrophy of the thyroid gland (× 1,200). Note hypertrophy of the Golgi apparatus.

For 35 glands the diagnosis of parenchymal cellular hypertrophy of the thyroid with or without regenerative hyperplasia was made (table 1). In 3 glands adenomatous tissue was present. The Golgi apparatus was found to be enlarged in 34 of these 35 glands. In some it was greatly enlarged; in others, less so. Figure 1 shows a portion of the thyroid gland removed from a patient with exophthalmic goiter. Three weeks before operation this patient's basal metabolic rate was plus 73 per cent.

Iodine was given in the usual doses during this three weeks' preoperative period. While the patient's general condition improved with the lowering of the basal metabolic rate, it will be seen in figure 1 that definite cellular hypertrophy is still present and that the Golgi apparatus is markedly enlarged. The one gland in this series on which the diagnosis of parenchymal cellular hypertrophy of the thyroid gland was made and in which no hypertrophy of the Golgi apparatus was seen will be considered later.

Adenomatous Thyroid Gland.—Adenoma of the thyroid gland may be single, but usually it is multiple. Invariably, various types of degeneration may be seen in the adenoma. Some adenomas are encapsulated and anatomically demarcated from the surrounding colloid thyroid tissue in which they are situated. They contain fetal as well as colloid follicles. In cases in which adenomatous thyroid tissue produces clinical hyperthyroidism, hypertrophy of the cells lining the follicles in the adenomas is observed in a third of the cases. In such an event the process is spoken of as "intra-adenomatous hypertrophy," and columns of high cuboid cells are seen in the follicles, with infolding papillary projections of the epithelium, which are similar in appearance to the generalized process in parenchymal cellular hypertrophy of the thyroid gland. The colloid thyroid tissue surrounding the adenoma in this condition does not have hypertrophic epithelial changes. In approximately two thirds of the cases in which the diagnosis of adenomatous goiter with hyperthyroidism is made intra-adenomatous cellular hypertrophy is not present. In cases of adenomatous goiter, then, the presence or absence of clinical hyperthyroidism cannot always be correlated with the pathologic observations. To state the matter in other words: Many adenomatous thyroid glands producing clinical hyperthyroidism will not show evidence of cellular hypertrophy in the adenoma.

We have mentioned that diffuse parenchymal cellular hypertrophy involving extrinsic thyroid tissue may occur in a nodular goiter with or without intra-adenomatous hypertrophy. Such a condition is clinically and pathologically different from adenomatous thyroid with hyperthyroidism and essentially similar to the parenchymal cellular hypertrophy of the thyroid which does not contain adenomas.

Forty thyroid glands containing adenomatous tissue or adenomas were studied. In 3 of these diffuse hypertrophic changes were present in the extra-adenomatous tissue, and they have already been considered in the parenchymal cellular hypertrophy group. Eight of the 37 adenomatous thyroid glands under consideration showed intra-adenomatous hypertrophy (table 1). In 7 of these 8 glands the Golgi apparatus was found to be enlarged. The remaining 29 glands showed

no intra-adenomatous hypertrophy, although the Golgi apparatus was enlarged in 17.

It at once became evident that a large number of adenomatous thyroid glands with enlargement of the Golgi apparatus do not exhibit cellular hypertrophy. As we will show, many of these glands were producing hyperthyroidism when they were removed. No enlargement of the Golgi apparatus in the colloid thyroid tissue extrinsic to the adenoma was found in the glands studied from this standpoint. The number of glands

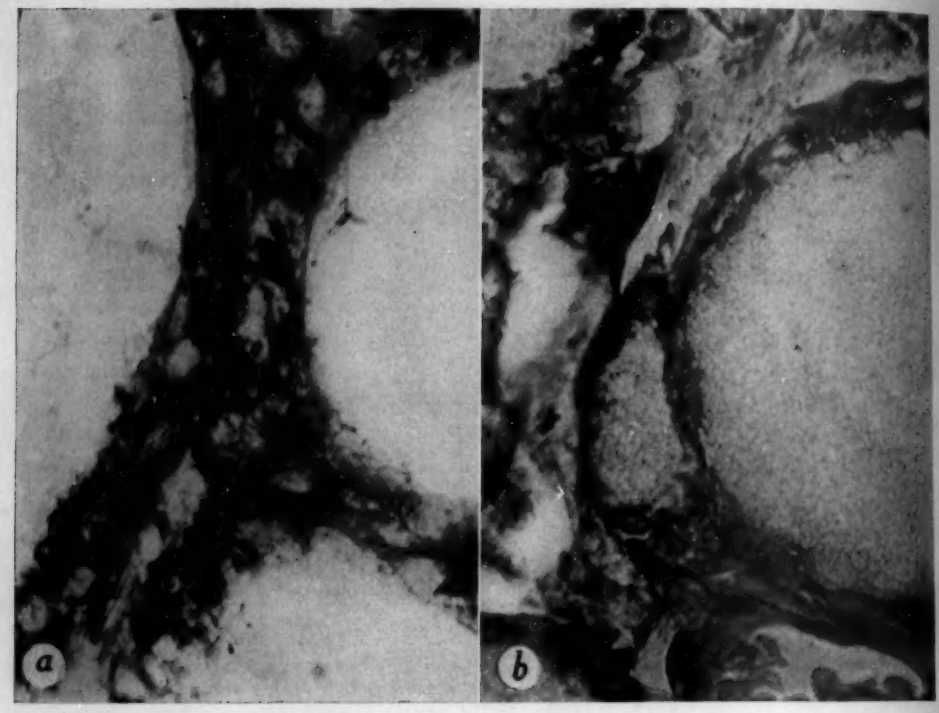

Fig. 2.—(*a*) Adenoma of the thyroid gland of a patient with hyperthyroidism, showing hypertrophy of the Golgi apparatus (×1,200). (*b*) Thyroid tissue extrinsic to the adenoma shown in *a;* the Golgi apparatus is not hypertrophied here.

studied in respect to this point of the size of the Golgi apparatus in the extra-adenomatous tissue we do not feel is sufficient to make the observations conclusive. This most interesting aspect of the study requires further work. Figure 2 *a* shows the Golgi apparatus in an adenoma of a thyroid gland removed from a patient with hyperthyroidism. The apparatus is dense and large and comparable to that seen in the gland with diffuse parenchymal hypertrophy. In certain instances

the enlarged Golgi apparatus in the adenoma appears to have a shape different from that seen in the thyroid presenting parenchymal cellular hypertrophy. This observation is now being subjected to further study. In figure 2 *b* the Golgi apparatus in the extra-adenomatous tissue of the same gland is seen to be small and difficult to define with certainty.

Colloid Thyroid Gland.—Only 1 specimen of colloid goiter was obtained, this condition being rarely encountered today.[6] Microscopic examination of a colloid goiter reveals a series of follicles lined with flattened or low cuboidal epithelium and containing abundant colloid. Okkels [4a] found the Golgi apparatus in this type of gland to consist of a fine strand, situated usually on the border of the nucleus which is toward the lumen. It was impossible to prepare suitable photomicrographs from our single specimen. In the very inactive cell of the colloid goiter, small strands of silver are seen with great difficulty. In many cells the Golgi apparatus unquestionably could not be seen; in fact, from our studies it seems that the apparatus may be observed easily and distinctly only when it is hypertrophied.

CORRELATION OF THE CLINICAL, HISTOLOGIC AND CYTOLOGIC OBSERVATIONS

While our series of cases is small, certain findings seem significant.

Parenchymal Cellular Hypertrophy of the Thyroid Gland.—The 35 thyroid glands on which the pathologic diagnosis of parenchymal cellular hypertrophy was made were removed from patients who had exophthalmic goiter clinically (table 2). As we have stated, the administration of compound solution of iodine preceded operation in each case. Usually, iodine was given for a period of from seven to fourteen days before operation; in some cases, however, treatment was prolonged over several months. In many of the cases in which the gland was removed at operation the pathologic diagnosis of parenchymal cellular hypertrophy was qualified by the statement that little cellular hypertrophy was left. As we have indicated, the diagnosis of hypertrophy of the thyroid gland associated with the disease exophthalmic goiter does not entirely depend on the degree of cellular hypertrophy but may be made from the general morphologic appearance of the gland even though marked regressive changes have occurred under iodine medication. All these glands except one had enlargement of the Golgi apparatus in the cells lining the follicles. This gland was removed from a patient who had been receiving iodine for five months before operation. On microscopic examination there was little evidence of cellular hypertrophy left.

From our study of this group of thyroid glands with parenchymal cellular hypertrophy it appears that cellular regression is accompanied

by a decrease in the size of the Golgi apparatus. In many of the follicles of the glands removed from patients with exophthalmic goiter who had been treated with iodine the cells showed marked regression and are flattened. In these cells no enlarged Golgi apparatus can be seen. This seems logical and explains our findings in the single case of exophthalmic goiter in which no enlarged Golgi apparatus was found in the cells of the gland removed at operation. In the majority of cases, however, areas are found with cells showing enlargement of the Golgi apparatus. We cannot agree with Okkels' statement that the size of the Golgi apparatus is not altered by iodine medication even though cellular regression is marked. We do note, however, that the enlargement of the apparatus in a thyroid gland of the hypertrophic parenchymal type is a more prominent feature than the cellular hypertrophy in the glands from certain patients who have obtained marked benefit from iodine therapy. It must

TABLE 2.—*Clinical Diagnoses and Microscopic Observations in Seventy-Three Cases of Thyroid Disease*

Clinical Diagnosis	Cases	Cases in Which Cellular Hypertrophy Was		Cases in Which Golgi Apparatus Was	
		Present	Absent	Enlarged	Not Enlarged
Exophthalmic goiter	35	35	..	34	1
Adenomatous goiter with hyperthyroidism	21	7	14	19	2
Adenomatous goiter without hyperthyroidism	16	1	15	5	11
Colloid goiter	1	..	1	..	1

be remembered, however, that administering iodine seldom is sufficient to restore normal metabolic conditions, and cellular hyperfunction is present in practically all of the glands removed at operation.

Adenomatous Thyroid Glands.—Twenty-one of the 37 adenomatous thyroid glands in this study were removed from patients with hyperthyroidism as determined by the usual methods (table 2). Of these 21 glands, only 7 showed intra-adenomatous cellular hypertrophy. On the other hand, the Golgi apparatus was found to be enlarged in 19, and in all these the enlargement was definite. These observations suggest that the size of the Golgi apparatus is a more delicate method of estimating cellular function than the usual histologic method. In 2 cases no enlargement of the Golgi apparatus was seen in the sections obtained for study.

Sixteen of the adenomatous thyroid glands were obtained at operation in cases in which the clinical diagnosis of adenomatous goiter without hyperthyroidism had been made (table 2). Figure 3 shows a portion

of an adenoma removed from a patient without clinical hyperthyroidism. The Golgi apparatus is enlarged and gives evidence of increased cellular function in this adenoma. The finding of enlargement of the Golgi apparatus in 5 of 16 of these cases of adenomatous goiter without hyperthyroidism (table 2) is of especial interest, and although the group is small, it suggests that hyperfunction of certain portions of the adenomatous thyroid may occur without producing a sufficient degree of hyperthyroidism to permit its recognition with certainty.

Fig. 3.—Adenoma of the thyroid gland of a patient without clinical hyperthyroidism, showing hypertrophy of the Golgi apparatus (× 1,200).

COMMENT

From the foregoing study of 73 cases of goiter, it seems true that the Golgi apparatus of the thyroid cells are of different sizes under varying conditions of activity and in various types of thyroid disease. In the cells of the normal gland and in the cells of the colloid thyroid Okkels [48] found the Golgi apparatus to be a small filament. Our observation in flattened cells which histologically appear only normally functioning or less so is that the Golgi apparatus cannot be clearly seen. The contrast between such cells, however, and those of definitely hyper-

functioning tissue, even under low power magnification, is so striking that one can immediately differentiate the two. When the Golgi apparatus is enlarged, it is easily seen (figs. 4 *a* and *b*, 5 *a* and *b* and 6), and the enlarged Golgi apparatus in hyperfunctioning tissue has a striking appearance.

We have found the Golgi apparatus enlarged in practically all the glands showing parenchymal hypertrophy removed from patients with exophthalmic goiter (34 of 35). Okkels [48] stated that he invariably found

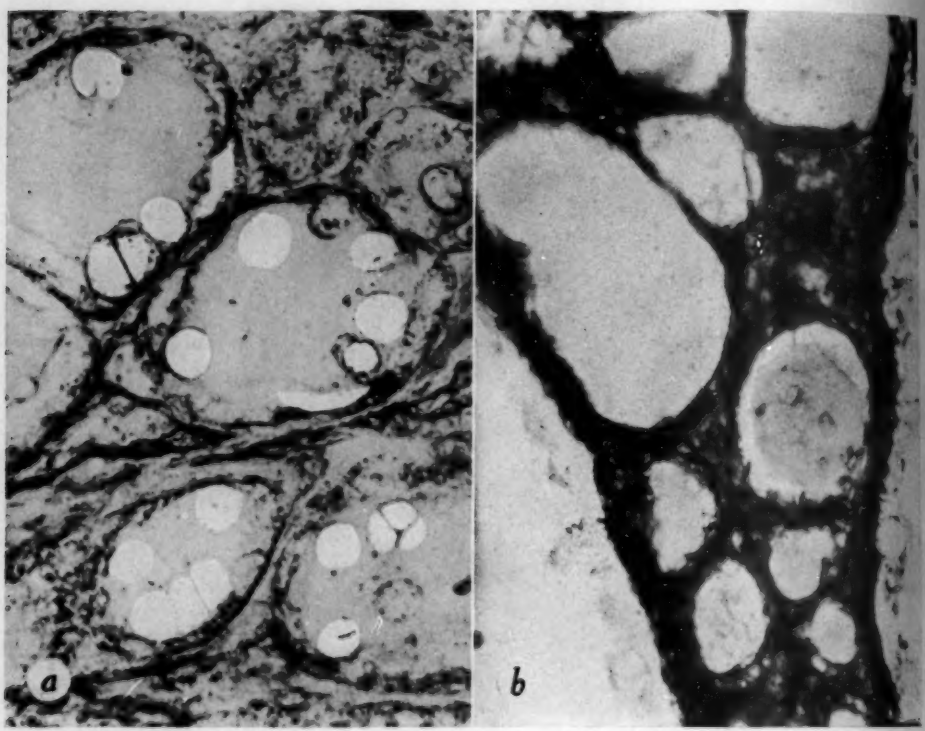

Fig. 4.—(*a*) Colloid thyroid tissue with no hypertrophy of the Golgi apparatus (× 485). (*b*) Colloid thyroid tissue extrinsic to a hyperfunctioning adenoma, with no hypertrophy of the Golgi apparatus (× 485).

enlargement of the apparatus of the thyroid cell in the presence of exophthalmic goiter, even after almost complete return of the gland to a state cytologically resembling colloid thyroid in the patient who had received iodine for some time. As a result, he was of the opinion that there is constant cellular hyperfunction and that iodine does not affect cellular function but rather effects an accumulation of colloid in the follicle by some other mechanism. This was also the observation of

Gilman,[4b] who recommended abandoning the use of iodine on the basis of these cytologic observations.

It is indeed hard to subscribe to these conclusions in the light of certain considerations. Obviously, in the majority of patients who have been operated on but who are still suffering from hyperthyroidism the basal metabolic rate is elevated. The cells are therefore still hyperfunctioning.

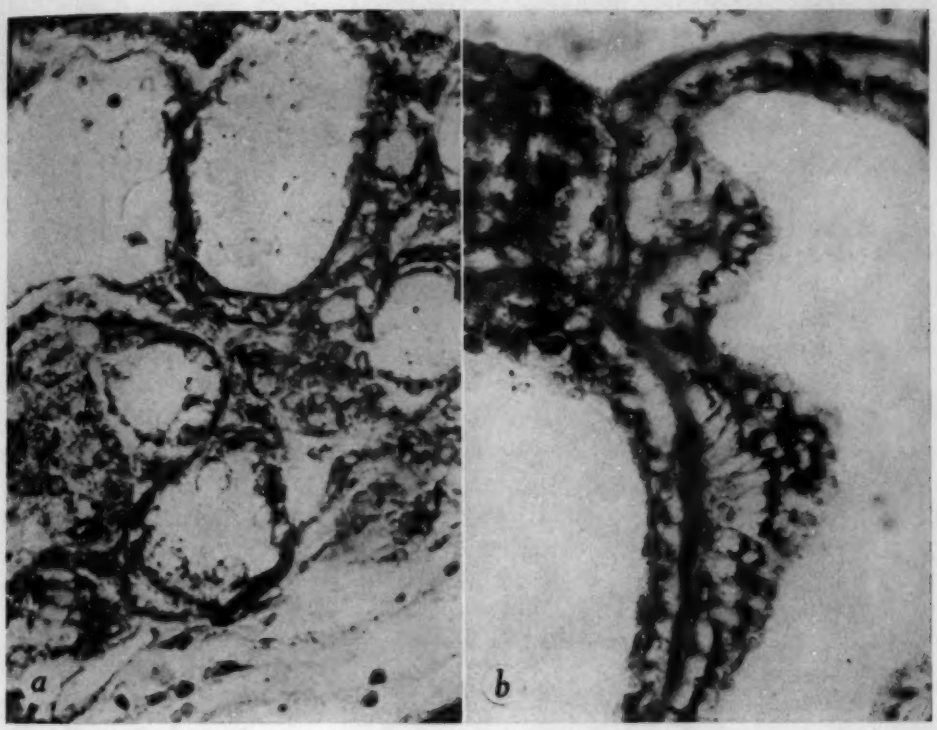

Fig. 5.—(*a*) Adenoma of a thyroid without cellular hypertrophy removed from a patient without hyperthyroidism; the Golgi apparatus is not hypertrophied (× 485). (*b*) Parenchymal cellular hypertrophy of the thyroid; the Golgi apparatus is hypertrophied (× 485).

The Golgi apparatus is enlarged and appears as a dense black mass in the cells of the adenomatous tissue which is hyperfunctioning and producing clinical hyperthyroidism. In 19 of 21 glands evidence of hyperfunction in the adenoma was obtained by the finding of a hypertrophied Golgi apparatus. Sufficient cellular hypertrophy to be considered evidence of hyperfunction could be found in only 7 of these glands. While the number of cases studied does not allow us to say what the exact correlation is between a recognizable degree of hyperthyroidism

and enlargement of the Golgi apparatus in adenoma of the thyroid, the correspondence seems fairly high, and enlargement of the apparatus is more definite evidence of hyperthyroidism than can be obtained from an estimation of cell size by the usual histologic methods routinely employed in the pathologic laboratory.

The finding of enlargement of the Golgi apparatus in the adenomatous tissue of thyroid glands not producing hyperthyroidism is of special interest. We occasionally find evidence of cellular hypertrophy in adeno-

Fig. 6.—Adenoma of a thyroid removed from a patient with hyperthyroidism; the Golgi apparatus is hypertrophied (× 485).

mas removed at operation from patients without clinical hyperthyroidism. In the case of nodular goiter the clinical diagnosis of hyperthyroidism is sometimes difficult to make, and it may in the final analysis depend on whether the basal metabolic rate is elevated above some arbitrary figure, such as plus 15 per cent. Boothby [15] expressed the opinion that there are, however, a considerable number of patients whose symptoms can-

15. Boothby, W. M.: Unpublished data.

not be definitely classified either as typical or as severe enough to warrant the diagnosis of hyperthyroidism. The basal metabolic rate may be from five to fifteen points above the patient's own mean, which, of course, may be definitely below the standard mean, which is the average of many persons. These persons may be suffering from mild degrees of hyperthyroidism, which may be extremely difficult to recognize with certainty. Hertzler [16] expressed the opinion, based on clinical observation, that a large number of goiters are toxic without giving evidence of it from the basal metabolic rate. It has been the observation of Haines and W. A. Plummer [17] for a long time that many patients with adenomatous goiter without hyperthyroidism feel immeasurably improved after thyroidectomy.

There is, then, considerable indication clinically that mild hyperthyroidism is present in a large group of persons with adenomatous goiter in spite of the fact that the condition frequently cannot be classified as hyperthyroidism. Boothby [15] recorded some evidence of this mild activity of adenomatous thyroid tissue. From the standpoint of the pathologic anatomy of the adenomatous thyroid gland, it is a reasonable conception that the nondegenerative portions of the adenoma are functioning beyond the capacity of normal cells or of the cells in thyroid tissue extrinsic to the adenoma. Boothby suggested a reasonable explanation for the physiologic activity of the adenomatous gland which will allow a presumption of increased production of thyroxin by the adenoma in the patient with adenomatous goiter without resulting in hyperthyroidism. He has postulated that in the event of increased production of thyroxin by the adenoma a mechanism which regulates the normal total production of thyroxin per day (0.4 mg.) decreases the activity of the extrinsic or normal tissue in the gland. This process of compensatory functional retardation of the extrinsic glandular tissue may be exhausted by an ever increasing size of the abnormally functioning adenomas. In such an event clinical hyperthyroidism may result. With this concept of the diseased adenomatous goiter in mind, it becomes clear that the development of hyperthyroidism may be slow. A concomitant degeneration of certain portions of the adenoma is another factor in delaying the onset of increased production of thyroxin. These postulates with regard to adenomatous goiter present a picture of a process progressive toward the hyperthyroid state.

SUMMARY

A study of the Golgi apparatus of the thyroid gland has been made in 73 thyroid glands from patients who came to operation for goiter. Hypertrophy of the Golgi apparatus was found in 34 of 35 glands

16. Hertzler, A. E.: Surgical Pathology of the Thyroid Gland, Philadelphia, J. B. Lippincott Company, 1936.

17. Haines, S. F., and Plummer, W. A.: Personal communication to the authors.

removed from patients with exophthalmic goiter and in 19 of 21 glands removed from patients with adenomatous goiter with hyperthyroidism. Hypertrophy of the apparatus was also found in 5 of the 16 adenomatous goiters removed from patients who were not judged to have hyperthyroidism. Appraising the size of the Golgi apparatus in the thyroid gland would therefore seem to offer a more delicate method of estimating cellular and glandular function than ordinary methods.

This study has purposely not been made on selected pathologic groups. Certain general information has been obtained which opens avenues for considerable study of the various types of goiter individually, using this method of demonstrating the Golgi apparatus. Unfortunately, the method of preparing and staining sections to demonstrate the Golgi apparatus is too time-consuming to be used in the routine pathologic diagnosis of surgical material.

CHEMOTACTIC PROPERTIES OF TUBERCULO-PHOSPHATIDE AND TUBERCULO-POLYSACCHARIDE

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It is well established that in vitro the tubercle bacillus exerts a positive chemotropic action on both human and animal polymorphonuclear neutrophils and that in experimental infections of animals there is early exudation of these cells into the infected regions. In a previous paper it was reported that tuberculoprotein when adsorbed on kaolin or charcoal strongly attracts human polymorphonuclear leukocytes.[1] The present study is concerned with the phosphatide and polysaccharide fractions of the tubercle bacillus.

METHODS

Tuberculophosphatide was obtained from the H-37 strain of human tubercle bacilli.[2] The polysaccharide[3] was prepared from strain A-10 and has properties similar to those of the crude polysaccharide described by Heidelberger and Menzel.[4] Both preparations belong in the group of water-alcohol-ether extracts of living tubercle bacilli which has been designated residue I.[5]

Both rabbit and human leukocytes were studied. Rabbit polymorphonuclear leukocytes were secured by injecting sterile physiologic solution of sodium chloride into the peritoneal cavity and were suspended in homologous plasma. Human polymorphonuclear leukocytes were obtained from finger blood of presumably healthy adults.

The method employed to study the chemotropism of the leukocytes has been described in detail elsewhere.[1] A small amount of the substance to be tested was placed on a clean glass slide. A drop of plasma-leukocyte suspension or finger blood was placed on a cover slip and spread over the test substance, which thus

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1. Wartman, W. B.: Arch. Path. **26**:694, 1938.

2. Crowder, J. A.; Stodola, F. H.; Pangborn, M. C., and Anderson, R. J.: J. Am. Chem. Soc. **58**:636, 1936.

3. Anderson, R. J.; Reeves, R. E., and Stodola, F. H.: J. Biol. Chem. **121**:649, 1937.

4. Heidelberger, M., and Menzel, A. E. O.: J. Biol. Chem. **118**:79, 1937.

5. Sabin, F. R., and Joyner, A. L.: J. Exper. Med. **68**:853, 1938.

formed a small target on the slide. Because the phosphatide and polysaccharide are soluble in blood plasma it was necessary to use as a target some chemotactically inert substance saturated with the test solution. The substances employed were kaolin and titanium dioxide. Control experiments were made on the same slide, with pure washed kaolin or titanium dioxide being used as targets.

The fraction to be tested was dissolved in fresh triple-distilled water and allowed to come into contact with kaolin or titanium dioxide, which was then used as the target. Each sample of kaolin or titanium was tested to determine whether or not phosphatide or polysaccharide was actually present, and only those preparations were used which contained the desired fraction. The movements of leukocytes near the target were observed under the microscope in a warm chamber at 37 C., and the course of each cell was recorded with the aid of a drawing ocular at one minute intervals for a period of ten minutes. The index of chemotropism was defined as the ratio between the net approach of a cell either toward or away from the target to the actual distance traveled by the cell. In each group of experiments the results were expressed as the mean chemotactic index of all the cells observed.

TABLE 1.—*Chemotactic Properties of Phosphatide from the Tubercle Bacillus*

	Experiment		Control	
Substance Tested	Cells	Mean Chemotactic Index with Standard Error	Cells	Mean Chemotactic Index with Standard Error
Pure phosphatide	20 human leukocytes	-0.09 ± 0.06	39 human leukocytes	-0.03 ± 0.07
Pure phosphatide	38 rabbit leukocytes	$+0.15 \pm 0.04$	122 rabbit leukocytes	-0.05 ± 0.01
Phosphatide on titanium dioxide	111 rabbit leukocytes	$+0.43 \pm 0.03$	137 rabbit leukocytes	$+0.02 \pm 0.06$
Phosphatide on kaolin	110 rabbit leukocytes	0.00 ± 0.01	88 rabbit leukocytes	-0.08 ± 0.04

RESULTS

Three different experiments were carried out with the phosphatide. In the first the undissolved powdered phosphatide was used as the target. In the second and third experiments titanium dioxide and kaolin moistened with dilute solutions of phosphatide were employed as targets.

When powdered tuberculophosphatide was used as the test material, most of the leukocytes in the vicinity of the target showed a decrease in motility and soon became immobile (table 1). Shortly many of the cells died. The cells which showed motion moved slowly, and the chemotactic index was not significantly different from the value obtained for leukocytes observed in fields remote from the target.

When tuberculophosphatide and titanium dioxide were used, the leukocytes were weakly attracted to the target. The chemotactic index was $+ 0.43 \pm 0.03$. Control experiments with titanium dioxide gave a chemotactic index of approximately zero. Similar experiments in which kaolin was used as an opaque substance gave a chemotactic index of

0.00 ± 0.01, indicating random motion. The kaolin control experiments likewise showed random motion.

The results of the experiments with the tuberculopolysaccharide in which kaolin was used as an inert substance indicate that this fraction weakly repelled the leukocytes (table 2). Again, the kaolin control showed random motion.

COMMENT

These experiments indicate that concentrated, undiluted tuberculophosphatide is toxic for both human and rabbit leukocytes, since it reduces the motility of the cells and finally kills them. When, however, the phosphatide is diluted, weak attraction is observed. The polysaccharide, on the other hand, elicits weak negative chemotaxis.

It thus seems probable that in vitro the protein fraction of the tubercle bacillus is chiefly responsible for attraction of polymorpho-

TABLE 2.—*Chemotactic Properties of Polysaccharide from the Tubercle Bacillus*

	Experiment		Control	
Substance Tested	Cells	Mean Chemotactic Index with Standard Error	Cells	Mean Chemotactic Index with Standard Error
Polysaccharide on kaolin.....	132 human leukocytes	−0.34 ± 0.05	120 human leukocytes	+0.05 ± 0.04
Polysaccharide on kaolin.....	71 rabbit leukocytes	−0.34 ± 0.06	37 rabbit leukocytes	+0.02 ± 0.10

nuclear neutrophils.[1] As far as could be determined this fraction is not harmful to the cells. The polysaccharide, on the other hand, does not strongly attract neutrophils and may even weakly repel them. The action of the phosphatide, however, is complicated by its markedly injurious effect. Probably polymorphonuclear leukocytes migrate toward the phosphatide but are killed before reaching it.

Dixon, McCutcheon and Czarnetsky [6] obtained similar results with fractions prepared from a strain of hemolytic streptococci. They showed that the fraction which produces antibodies in experimental animals (protein-carbohydrate complex) also causes positive chemotropism of rabbit leukocytes, while a carbohydrate fraction and a "protein-free, nonantigenic, crystalline stabile hemolysin" elicit no chemotropism. Under different experimental conditions Sabin [7] reported that all the fractions of the tubercle bacillus cause an outpouring of leukocytes when they are injected into guinea pigs (subcutaneous, intracutaneous

6. Dixon, H. M.; McCutcheon, M., and Czarnetsky, E. J.: Am. J. Path. **13**:645, 1937.

7. Sabin, F. R.: Physiol. Rev. **12**:141, 1932.

and intraperitoneal injections). Sabin noted that the medium in which the fractions were suspended also caused emigration of leukocytes into the peritoneal cavity, but to a lesser degree. This discrepancy in results is at present unexplained but may be due to the difference in experimental conditions.

SUMMARY

The chemotactic properties of a phosphatide and a polysaccharide fraction of the tubercle bacillus have been studied in vitro. Under the conditions of the experiments it was found that the tuberculophosphatide in concentrated form is toxic for both human and rabbit neutrophils, but in suitable dilution it causes weak attraction of these cells. The tuberculopolysaccharide causes weak negative chemotropism of the leukocytes.

HISTOLOGIC CHANGES IN THE RENAL ARTERIOLES OF HYPERTENSIVE RABBITS

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In a recent review Goldblatt [1] stated that in renal hypertension, "if there is no accompanying renal insufficiency, the kidneys show no significant gross or microscopic changes detectable by usual methods." He added that the changes in the afibrillar cells of the preglomerular arterioles reported by Goormaghtigh and Grimson [2] are interesting as the only changes occurring in hypertension, but stated that their significance awaits elucidation. Goormaghtigh [3] more recently reported that the juxtaglomerular apparatus of normal rabbits contains chiefly afibrillar cells which exhibit a normal glandular cycle of vacuolation and acidic and basic granulation. Moreover, he reported that in 3 rabbits rendered hypertensive by the Drury technic there was an increase in number and size of these granular cells, an increase of afibrillar cells and formation of granular cells in the preglomerular arterioles. Goormaghtigh suggested that the afibrillar cells are responsible for arteriolar tone and that the granular cells are the source of the pressor substance in hypertension. In this report we wish to offer corroboration and extension of Goormaghtigh's findings.

MATERIAL AND METHODS

This report is based on a study of kidneys from 39 rabbits, on which blood pressures were determined by the abdominal cuff method of McGregor.[4] Of this group, 14 rabbits were "normal" or untreated; 12 received injections of aspartic acid, either intramuscularly or intravenously; 7 had one renal artery partially ligated by the Drury [5] technic, and 6 had one or both renal arteries partially ligated by a modification of Goldblatt's [1] method. Eight of the "normal" or control rabbits

This work was supported in part by a grant from the Committee on Scientific Research of the American Medical Association.

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1. Goldblatt, H.: Am. J. Clin. Path. **10**:40, 1940.
2. Goormaghtigh, N., and Grimson, K. S.: Proc. Soc. Exper. Biol. & Med. **42**:227, 1939.
3. Goormaghtigh, N.: Proc. Soc. Exper. Biol. & Med. **43**:688, 1939.
4. McGregor, L.: Arch. Path. **5**:630, 1928.
5. Drury, O. R.: J. Exper. Med. **68**:695, 1938.

had blood pressures ranging from 80 to 120 mm., and 6 exhibited mild spontaneous elevations of between 20 and 30 mm. above a maximum normal pressure of 120 mm. In the group which received injections of aspartic acid, 7 rabbits had normal pressures and 5 showed elevations of from 20 to 30 mm. The remaining rabbits, on which partial ligation of the renal artery was done by either the Drury or the Goldblatt procedure, exhibited elevated pressures, ranging from 150 to 240 mm.

The kidneys were removed immediately after the rabbits were killed and fixed in Zenker's solution prepared with formaldehyde, sectioned 4 to 5 microns in thickness and stained according to Goldner's [6] modification of Masson's trichrome stain.

RESULTS

In the 15 rabbits which had normal blood pressures throughout the period of observation (8 normal and 7 treated) the juxtaglomerular apparatus consisted of a few afibrillar or paucifibrillar cells and varying numbers of sparsely granular cells (*A*, fig. 1). The granulations in the latter cells gave a basophilic reaction in some and an acidophilic reaction in others. The acidophilic granular cells ("A") were usually, although not invariably, present in greater numbers than the basophilic granular cells ("B"). From one to three densely granular "A" cells were seen in the juxtaglomerular apparatus of some glomeruli and none in others. The "B" cells were invariably sparsely granular. Most of the so-called afibrillar cells of Goormaghtigh were found to contain faint basophilic stippling; however, these cells appeared much lighter than the typical "B" cells. Situated near the nucleus of each granular cell was a clear vacuole-like area containing one or more spherical or irregularly shaped, darkly staining structures (*A*, fig. 1). This complex, which will henceforth be referred to as the vacuolar complex, resembled a similar structure found in the basophilic cell of the pituitary gland. In the "B" cells and sparsely granular "A" cells this complex was small and often indefinite; however, in the densely granular "A" cells it was larger and quite distinct. The afibrillar and paucifibrillar cells were devoid of this complex. In these normal kidneys, as well as in those of the experimental rabbits to be described subsequently, the granular and afibrillar cells were located almost exclusively in the outer cortex.

A striking change was seen in the juxtaglomerular apparatus and renal arterioles of rabbits with blood pressures of 170 mm. or over (M_8, M_{15}, H_1, H_2, H_3, 163, 150). In these animals, particularly M_8, M_{15} and H_2, there was a marked increase in size and number of "A" cells in the juxtaglomerular apparatus (*B* and *C*, fig. 1), apparently at the expense of the afibrillar and "B" cells, for the latter were reduced in number as compared with the normal. The density of granulation in many of the "A" cells was greatly augmented, giving them a brilliant red granular appearance, which made them readily visible under magnifications of 100 diameters. Some of the "A" and "B" cells in these

6. Goldner, J.: Am. J. Path. **14**:237, 1938.

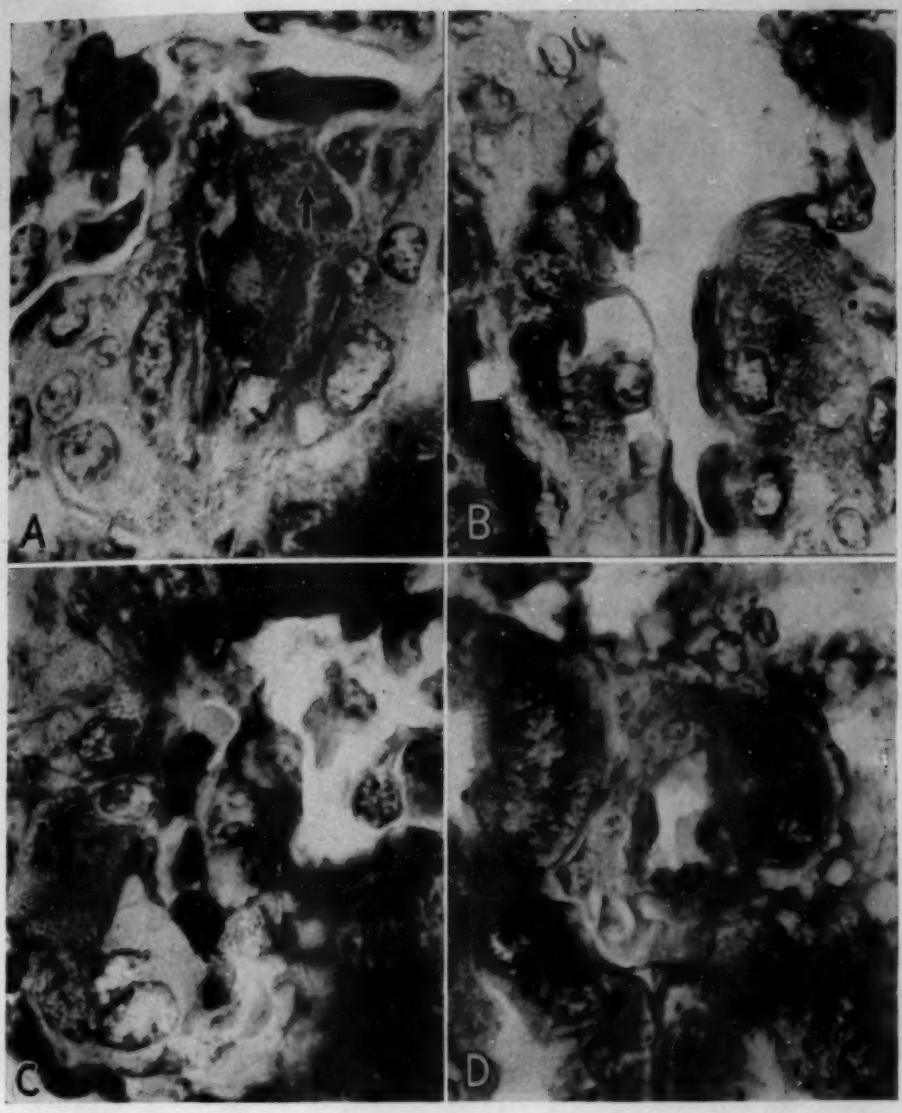

Figure 1

Photomicrographs; × 1,635. The glomerulus in all photographs of the juxtaglomerular apparatus lies in the field above that shown.

A, juxtaglomerular apparatus of control rabbit 7 (blood pressure, 110 mm.). On the right are acidophilic granular cells, most of which are densely granular. A vacuolar complex is indicated by the arrow. On the left are several sparsely granular "B" cells. In the lower cell of this group some of the granules are acidophilic.

B, juxtaglomerular apparatus of experimental rabbit M15 (partial ligation of both renal arteries, Goldblatt type; blood pressure, 240 mm.). All cells of this juxtaglomerular apparatus are of the acidophilic granular type. The three or four cells at the upper left are sparsely granular. Note the vacuolation of the large granular subendothelial cell on the left.

C, juxtaglomerular apparatus of experimental rabbit M8 (partial ligation of one renal artery, Goldblatt type; blood pressure, 175 mm.). The large clear binucleate cell with faint granulations at the lower left is a "B" type, but a few of the granules stain acidophilic. The granular cell to the left and above this one is also binucleate, but the nuclei do not show in this plane. Except for the light cells in the lower center, all other cells are of the "A" type.

D, cross section through an arteriole of rabbit M15 (see note on *B* for protocol). Note the large granular subendothelial "A" cell.

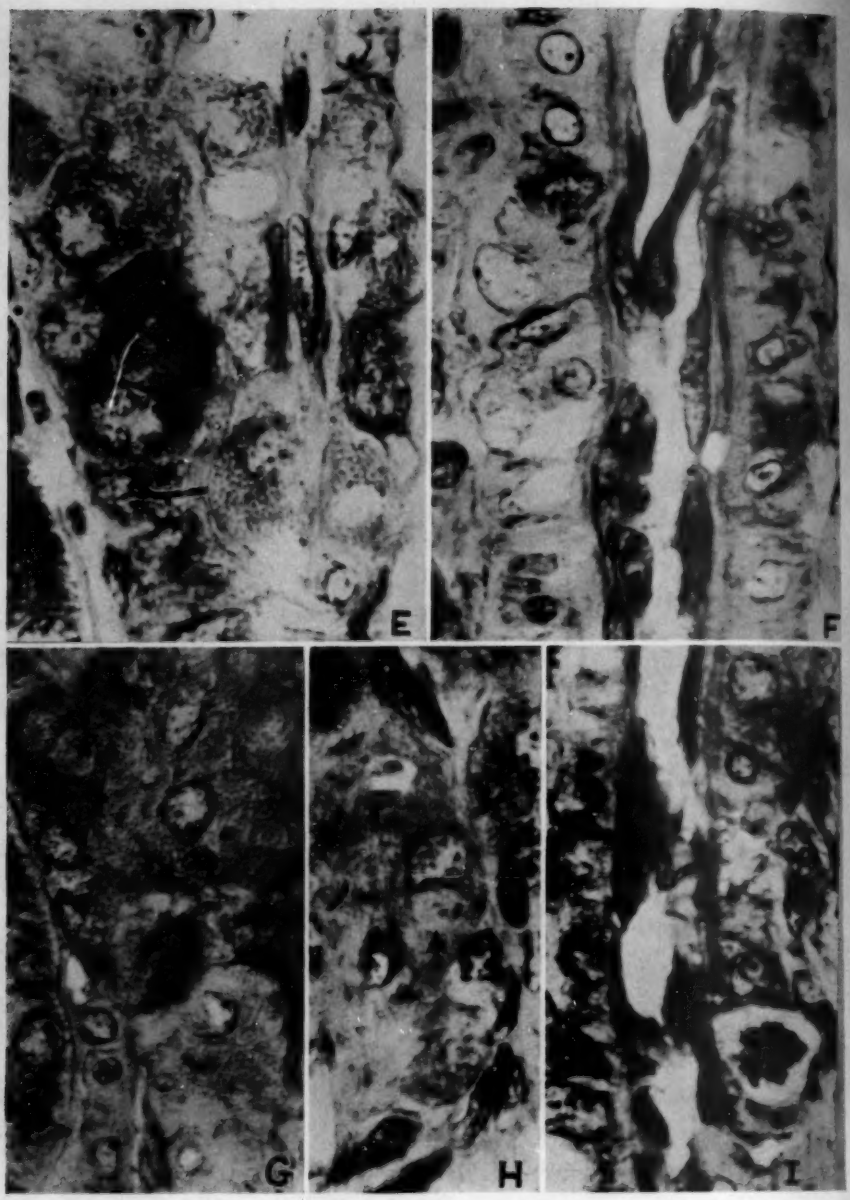

Figure 2

Photomicrographs; × 1,635.

E, longitudinal section through a branching arteriole of rabbit M 15 (see note on *B* for protocol). All the granular cells present are acidophilic in type. Note the gradation in density of granulation.

F, section through an arteriole of experimental rabbit H3 (partial ligation of one renal artery, Drury type; blood pressure, from 200 to 163 mm.). Note the large vesicular nucleus of the medial cell at the left of center and the variability in size and staining reaction of other medial cells. Typical-staining smooth muscle cells are seen at the extreme lower left.

G, section through a branching arteriole of rabbit M15 (see note on *B* for protocol). Note the large number of densely granular "A" cells.

H, acidophilic granular cells from glomerular arteriole of rabbit M15 (see note on *B* for protocol). The arrow indicates an enlarged vacuolar complex.

I, section through arteriole of rabbit H2 (Drury type; blood pressure, 186). Note the mitotic figure in the afibrillar cell.

animals were binucleate (*C*, fig. 1). The vacuolar complex in the "A" cells was enlarged and prominent (*H*, fig. 2), and vacuolation of the cytoplasm was more extensive (*B*, fig. 1). The "B" cells were not enlarged and differed from the normal only in some instances in which there was an apparent development of acidophilia in the granules, with occasional distinctly acidophilic granules. The preglomerular arterioles were markedly altered by the presence of densely granular and developmental stages of the "A" cells in the media, especially at the points of branching (*G*, fig. 2 and *D*, 1). In this location a complete transitional series from clear afibrillar to mature "A" cells was present (*E*, fig. 2). Granular cells were never present in the arterioles of rabbits with normal pressures. Scattered singly in the media were also cells with faint basophilic stippling. Furthermore, the number of typical

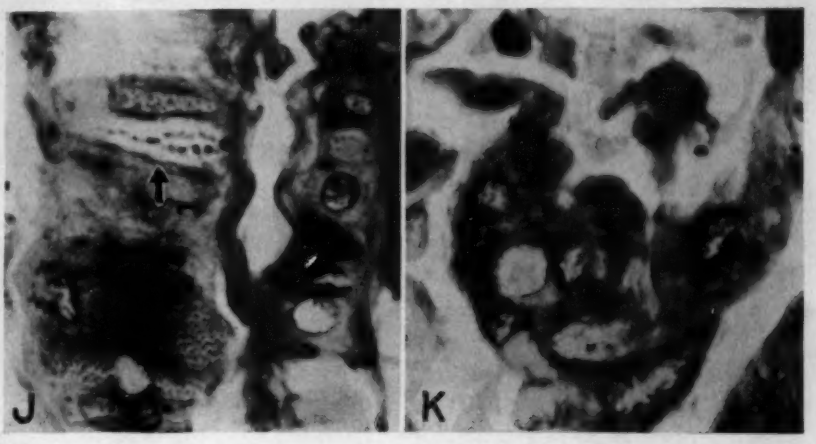

Figure 3

Photomicrographs; × 1,635.

J, longitudinal section through an arteriole of rabbit M8 (see note on *C* for protocol). The arteriole on the left is cut tangentially, and the lumen lies beneath the cells. Note the densely granular cells at the lower left; one contains a large vacuole. The arrow indicates a clear cell containing many fuchsinophilic bodies. On the right is the left wall of an adjacent arteriole. Most of these cells are granular or vacuolated.

K, juxtaglomerular apparatus of rabbit 163 (partial ligation of renal artery. Goldblatt type; blood pressure, 156 mm.). Note the muddy cytoplasm and the absence of distinct granulation in the juxtaglomerular cells.

staining smooth muscle cells was reduced, these apparently being replaced by afibrillar cells or cells of the granular series (*J*, fig. 3). In animal M_8, and to a lesser extent in the others, some of the clear cells contained spherical fuchsinophilic bodies, which are interpreted as indicating secretory exhaustion of granular cells of the "A" type (*J*, fig. 3).

Mitosis of afibrillar cells in the media was encountered only in animal H_1 (*I*, fig. 2), which, although high blood pressure developed in it, did not have even the normal number of granular cells in the juxtaglomerular apparatus. However, the number of afibrillar and paucifibrillar cells was greatly increased in this animal. In animals H_2, 150 and 163, in which blood pressures were elevated for periods ranging from four to twelve months, there were widespread tubular necrosis and fibrosis of glomeruli. The juxtaglomerular apparatus of the affected glomeruli in these animals was reduced in size and contained only darkly staining cells which were neither typical granular cells nor typical smooth muscle cells (*K*, fig. 3). "A" cells were present in the preglomerular arterioles and were increased in number in the juxtaglomerular apparatus of the few relatively normal glomeruli. In rabbit H_3 the juxtaglomerular apparatus contained increased numbers of "A" cells, and these cells were also present in the glomerular arterioles; however, the "A" cells contained very few acidophilic granules. The small number of densely or moderately granular cells and the large number of sparsely granular cells, in view of the blood pressure records of this animal, suggest a reversion of "A" cells to the afibrillar or paucifibrillar type. These afibrillar cells were abundant in the media of the arterioles (*F*, fig. 2). The blood pressure records of H_3 reveal an initial pressure of 200 mm. following removal of the normal kidney and a subsequent gradual drop over a period of three and one half months to 162 mm.

The changes in the juxtaglomerular apparatus and preglomerular arterioles were not so pronounced in animals with only moderate hypertension (140 to 160 mm.) following partial ligation of the renal artery. In these animals there was a moderate increase in number and size of "A" cells of the juxtaglomerular apparatus, but the granulation of these cells was not markedly increased. The size of the granules was, however, distinctly larger in many of these cells. The number of afibrillar and "B" cells seemed to be decreased, and "A" cells were rarely found in the arterioles. However, there was a distinct increase of afibrillar cells in the preglomerular arterioles, which is, perhaps, the most noticeable change in the kidneys of these animals.

The rabbits which exhibited mild spontaneous elevations of pressure, whether of the "normal" group or of the group receiving injections of aspartic acid, revealed only a very slight increase of "A" cells in the juxtaglomerular apparatus and a moderately increased number of afibrillar cells in the arterioles. The cause of these mild spontaneous elevations is the subject of another communication.[7]

COMMENT

Our observations on kidneys of normal and hypertensive rabbits suggest that the granular "A" cells in the juxtaglomerular apparatus

7. Dunihue, F. W.: Anat. Rec. (supp.) **73**:64, 1939.

and also in glomerular arterioles of hypertensive animals are secretory cells. The variation in the density of granulation of the "A" cells in normal animals and the increase in size as well as density of granulation in hypertensive rabbits support this conclusion. Further evidence is provided by the behavior of the vacuolar complex, which is usually enlarged and prominent in the densely granular "A" cells, especially in hypertensive rabbits. And finally the presence of a variable number of fuchsinophilic bodies in some of the clear cells of the preglomerular arterioles of acutely hypertensive rabbits is interpreted as indicating secretory exhaustion of granular cells. That the latter cells are probably formed from densely granular "A" cells is borne out by the finding of occasional fuchsinophilic bodies and enlarged acidophilic granules in some of the "A" cells. Therefore we concur with Goormaghtigh in holding these cells to be secretory.

If we accept the "A" cells as being secretory, their function as judged by location in the juxtaglomerular apparatus and preglomerular arterioles seems to be concerned with glomerular blood flow. The increase in size, number and secretory activity of the "A" cells in these locations in hypertensive rabbits further suggests such a function. Of course, it cannot be stated with certainty whether the activity of the "A" cells is cause or effect with respect to the elevated blood pressure. However, it seems reasonable on the basis of histologic evidence to assume, as does Goormaghtigh, that these cells produce a pressor principle. In this connection it is suggestive that the renal pressor substance (renin) is found only in extracts of the kidney cortex.[8] Under normal conditions the production of pressor substance, as judged by the appearance of the "A" cells, is probably not great. But in hypertensive animals these cells are increased in number and activity, forming glandular tissue, the total mass of which must be considerable. The products of secretion of such a mass of cells might conceivably affect the systemic blood pressure.

In both the normal and hypertensive kidney there is considerable evidence to indicate that the afibrillar, the "B" and the "A" cells form a developmental series. That there is a relationship between the "A" and "B" cells seems unquestionable. They both possess a vacuolar complex, and "B" cells have been observed which contain a few acidophilic granules. Although the nature of this relationship is at present not entirely clear, it seems that the "B" cells may form "A" cells. The afibrillar cells, although they do not possess a vacuolar complex, certainly give rise to "A" cells in the arterioles of hypertensive rabbits, as evidenced by the closely graded series from afibrillar to densely granular "A" cells. The evidence for the formation of "B" cells from afibrillar cells is not so clearcut, but this seems likely in view of the

8. Pickering, G. W., and Prinzmetal, M.: Clin. Sc. **3**:211, 1938.

gradations in granularity found in the afibrillar and "B" cells. We suggest that the afibrillar cells are the probable stem cells of the granular series. This is indicated by the increase of afibrillar cells in the arterioles of moderately hypertensive rabbits and the presence in the same location in acutely hypertensive animals of mature and developing granular cells. The fact that mitoses were observed only in afibrillar cells also argues for this interpretation. In view of this, we do not feel that afibrillar cells are directly concerned with maintaining local arteriolar tone as suggested by Goormaghtigh.

We have found no evidence to indicate the origin of the afibrillar cells in the juxtaglomerular apparatus, but when present in the arterioles these cells seem to have developed in locations ordinarily occupied by smooth muscle cells. This and the fact that the smooth muscle cells exhibit varying degrees of fibrillation suggest origin of afibrillar cells from smooth muscle. It is interesting to note in this connection that E. R. Clark and E. L. Clark[9] report the formation of smooth muscle cells from fibroblasts and the reversion of muscle cells to fibroblasts in their observations on capillary growth in transparent windows in rabbits' ears.

SUMMARY

The juxtaglomerular apparatus of the normal rabbit kidney contains afibrillar, basophilic granular and acidophilic granular cells which exhibit signs of cyclic secretory activity. The granular cells are present only in the juxtaglomerular apparatus of the normal kidney, but the afibrillar cells are present also, in small numbers, in the glomerular arterioles.

In hypertension, produced either by the Goldblatt or by the Drury technic, there is an increase in size and number of the acidophilic granular cells in the juxtaglomerular apparatus, and all three types of cells, especially the acidophilic granular, are present in the glomerular arterioles.

It is suggested that these three types of cells form a developmental series having a probable origin from smooth muscle cells, and that the acidophilic granular cells are the source of the renal pressor substance.

9. Clark, E. R., and Clark, E. L.: Am. J. Anat. **66**:1, 1940.

EFFECTS OF THYROID AND CALCIUM THERAPY ON THE GROWTH OF SARCOMA TRANSPLANTS IN THYROPARATHYROIDECTOMIZED RATS

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Much literature has accumulated in the last few years with reference to the effect of thyroid and parathyroid hormones on the susceptibility of animals to growth of cancer and of tumor transplants. Murohara [1] found that removal of the thyroid gland had a tendency to inhibit the growth of a transplantable rabbit sarcoma. Paik [2] found that in parathyroidectomized rats the rate of growth of the Flexner-Jobling rat carcinoma was decreased. Matsuoka [3] reported the opposite observations on a similar tumor.

It is difficult to evaluate the seemingly confirmatory and contradictory results of different authors relative to the influence of various biologic substances on the growth of tumors because of the tremendous variability in the types of tumors used and in the characteristics of the biologic preparations. Even though the tissues of two tumors may be histologically similar, their reaction to chemotherapy may be markedly different. Another factor is the hereditary background of the host. Many authors have failed to state whether or not litter mates were used as controls in their experiments.

The conflict in results which have been reported relative to tumor growth in relation to metabolism makes it highly desirable that more detailed studies be made.

From the Departments of Pathology and Physiology, Loyola University School of Medicine.

1. Murohara, N.: Tr. Jap. Path. Soc. **20**:658, 1930.
2. Paik, T. S.: Am. J. Cancer **15**:2756, 1931.
3. Matsuoka, S.: Tr. Jap. Path. Soc. **20**:657, 1930.

The animals used in this study were from our own colony of Wistar Institute albino rats, which is maintained on a diet of fox chow,[3a] furnished ad libitum, with bread and meat twice a week. At the age of 21 days they were weaned, and between the ages of 26 and 35 days their thyroid and parathyroid glands were removed. Thyroparathyroidectomy was carried out on the anesthetized animal. The neck was shaved and iodine applied to the depilated area. A midline incision was made, exposing the muscles on the anterior side of the neck, which were separated by blunt dissection, exposing the thyroid. The lower pole of the left lobe of the thyroid was then lifted with a small pair of hand forceps, and tension was applied while it was being dissected free from the underlying tissues. Precautions were taken not to injure the recurrent laryngeal nerve during the dissection. The lifting and blunt dissection were continued until the isthmus was freed from its tracheal attachments. The right lobe was dissected in the same manner as the left. The skin was closed with interrupted sutures and iodine applied to the wound.

Immediately after the animals had been operated on, litter mates of the same sex and as near the same weight as practical were divided into four groups (table 1). Groups 1 and 2 (18 males and 18 females, respectively) received a diet of fox chow only. Groups 3 and 4 (20 males and 18 females, respectively) received a diet consisting of 99.98 per cent fox chow and 0.02 per cent desiccated thyroid. The animals were kept on their respective diets throughout the course of the experiment. During the first one hundred and fifty days of the experiment a few deaths occurred in each experimental group. On the one hundred and fiftieth day the 59 remaining animals were found to be distributed as follows (table 1): groups 1 and 2, 13 males and 16 females, respectively, and groups 3 and 4, 18 males and 12 females, respectively. At this time the animals were anesthetized with ether, and two small pieces of viable spindle cell sarcoma, each about the size of a grain of wheat, were placed beneath the skin in each animal through a dorsal incision near the level of the first lumbar vertebra. The sarcoma was a highly malignant growth of spindle and amorphous cells, obtained from the uterus of a rat and kept alive by transplanting small pieces beneath the skin through successive generations.

The quantity of thyroid used in the replacement therapy was that previously established [4] as adequate to produce a significant beneficial effect on the host. Indexes to this beneficial effect were observed in weekly weights and in the femoral measurements obtained after the death of the animals. The weekly weights showed a stimulating effect on body growth at about the third week, which reached a maximum approximately seven weeks later. Beyond this time the thyroid-fed

3a. The formula of the fox chow is as follows:

Organic	Percentage	Inorganic	Percentage
Proteins	23	Iron	0.018
Fat	5	Magnesium	0.09
Fiber	4	Potassium	0.56
Ash	7	Silica	0.23
Nitrogen-free extract	54	Sodium	0.67
Moisture	7	Chlorine	0.68
		Phosphate	1.17
		Calcium	2.22
		Iodine	trace

4. Patras, M. C.: The Influence of Thyroid Feeding on the Skull Pattern of Thyroparathyroidectomized Rats, Thesis, University of Illinois Graduate School, 1939.

animals maintained their acquired stature. The second index, dealing with the size the femur, revealed a significant increase in the length of this bone in those animals which had received thyroid therapy.

The weights obtained on the day the tumors were transplanted revealed an average difference of 78 and 43 Gm. between the males and females, respectively, in favor of the groups receiving desiccated thyroid. The period of survival following the tumor inoculation varied from twenty-four to sixty-two days, with no significant difference between the sexes, nor was the longevity markedly influenced by the thyroid therapy.

The weekly weights obtained throughout the period of survival and at the time of death revealed a sharp increase beginning about two weeks after the tumor transplants were made. This rapid increase reached a maximum three to four weeks later, after which there was a slight decline.

As soon after death as practical, the tumors were removed and weighed. From these data was derived not only the average tumor weight but also the relation of the weight of the tumor to that of the host.

The average weight of tumor tissue per rat in the groups which did not receive thyroid therapy was found to be 69 Gm. for both males and females. The groups receiving desiccated thyroid yielded significantly more tumor tissue per rat than the groups which did not receive thyroid. The average tumor weight per rat on thyroid was found to be 94 Gm. for the males and 108 Gm. for the females. This difference of 25 to 39 Gm. in favor of an increase in tumor growth with thyroid therapy may be considered as corroborative of the view that thyroid stimulates new growth and susceptibility to cancer. Sugiura and Benedict [5] observed the rate of tumor growth to be very slow in animals fed a goitrogenic diet. Kosugi [6] claimed that thyroid deficiency exerted a marked inhibitory effect on the growth of the Kato rabbit sarcoma if this deficiency was established at the time of or before the tumor was transplanted. In general this work was confirmed by Nishida,[7] who added that substances which promote thyroid function seemed to accelerate tumor growth. Rohdenburg, Bullock and Johnston [8] obtained some evidence that thyroid deficiency decreased the susceptibility of animals to cancer. Bischoff and Maxwell,[9] on the other hand, were not able to find evidence that thyroparathyroidectomy influenced the rate of growth in a rat carcinoma.

A more detailed study of the data, taking into consideration the influence of thyroid on the host as well as on the tumor, does not support a conclusion that thyroid may have a specific effect on tumor growth. The average loss of body weight incurred by the animals between the time of the transplantation of tumor and their death was not found to be

5. Sugiura, K., and Benedict, S. R.: Am. J. Cancer **23**:541, 1935.
6. Kosugi, K.: Tr. Jap. Path. Soc. **27**:651, 1937.
7. Nishida, S.: Jap. J. Obst. & Gynec. **18**:195, 1935.
8. Rohdenburg, G. L.; Bullock, F. D., and Johnston, F. J.: Arch. Int. Med. **7**:491, 1932.
9. Bischoff, F., and Maxwell, J.: J. Pharmacol. & Exper. Therap. **46**:51, 1932.

significantly different. The weights of the females on fox chow at the time of death (table 1) after the tumors had been removed revealed a loss of 25 per cent, while those receiving thyroid lost 27 per cent. The same general relation was observed in the male groups.

Since the thyroid therapy superimposed on a fox chow diet did not reveal a significant specific effect on the growth of tumor transplants the question of mineral balance, which is known to be disturbed,[10] was

TABLE 1.—*Tumor Growth in Thyroparathyroidectomized Rats Fed Desiccated Thyroid*

Groups	Animals Operated On	Animals Operated on and Bearing Tumors	Average Weight of Animal at Beginning of Experiment	Average Weight of Animal on 150th Day	Average Weight of Animal and Tumor	Average Weight of Tumor	Average Weight of Animal Less Tumor
1 Males on fox chow	18	13	62	157	189	69	120
3 Males on fox chow and thyroid	20	18	62.5	235	290	94	196
2 Females on fox chow	18	16	57	132	167	69	98
4 Females on fox chow and thyroid	18	12	56	175	235	108	127

TABLE 2.—*Tumor Growth in Thyroparathyroidectomized Rats Fed Desiccated Thyroid and Calcium Carbonate*

Groups	Animals Operated On	Animals Operated on and Bearing Tumors	Average Weight of Animal at Beginning of Experiment	Average Weight of Animal on 150th Day	Average Weight of Animal and Tumor	Average Weight of Tumor	Average Weight of Animal Less Tumor
5 Males on fox chow and calcium	22	20	60.7	165	220	68	152
7 Males on fox chow, calcium and thyroid	18	15	61	280	348	89	259
6 Females on fox chow and calcium	15	15	55.2	159	211	67	144
8 Females on fox chow, calcium and thyroid	17	14	57.5	203	284	71	213

considered. Calcium therapy (table 2) was therefore instituted in a group of 72 thyroparathyroidectomized rats. Until the time of operation these animals were treated in the same manner as those previously described. After the operation they were divided into four groups (table 2). Groups 5 and 6 (22 males and 15 females, respectively) received a diet consisting of 99 per cent fox chow and 1 per cent calcium carbonate. Groups 7 and 8 (18 males and 17 females, respectively)

10. Tweedy, W. R.; Templeton, R. D.; Patras, M. C.; McJunkin, F. A., and McNamara R. W.: J. Biol. Chem. **128**:407, 1939.

received a diet comparable to that of groups 5 and 6 except for the addition of 0.02 per cent desiccated thyroid, which was substituted for an equivalent quantity of fox chow. Tumor transplants were made on the surviving animals one hundred and fifty days after the start of the experiment. Thirty-four days after these inoculations all animals were killed and the tumor tissue removed. This time of putting them to death was selected because the results obtained on groups without calcium therapy indicated that a maximum growth of host plus tumor was attained about thirty to thirty-five days after the transplantation of tumor.

A difference of 44 and 115 Gm. for the females and males, respectively, was observed in favor of thyroid feeding at the time the tumor transplants were made. Approximately this same difference, with a rapid rise in the weight, which began between the second and third week after the inoculations, continued until the close of the experiment. The animals were killed for the tumor tissue before the occurrence of a marked decline in the weight of the hosts resulted from the tumor growth.

A comparison of the quantities of tumor tissue obtained from the various groups revealed a slight stimulating effect of thyroid. The average weight of tumor tissue taken from the animals which did not receive thyroid was 68 and 67 Gm., respectively, for males and females, while the groups receiving thyroid therapy yielded 89 and 71 Gm. of tumor tissue, respectively, from males and females.

The stimulating effect of thyroid on tumor tissue loses its significance when its effect on the host is taken into consideration, since tumor tissue responds to thyroid therapy in practically the same manner as normal tissue.

SUMMARY

The influence of thyroid on tumor growth was proportional to its effect on the general metabolism, and the addition of 1 per cent calcium carbonate to the diet had no significant effect on the growth of the host or of the sarcoma transplant.

Case Reports

CONGENITAL ATRESIA OF THE PANCREATIC DUCT WITH CYSTIC FIBROSIS OF THE PANCREAS

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Anderson [1] recently reviewed 49 cases of congenital pancreatic steatorrhea; 27 of these cases were already recorded in the literature and 22 were reported for the first time. The pathologic alteration of the pancreas in this series was fairly uniform, consisting usually of a lesion best described as cystic fibrosis. Occasionally complete fatty atrophy was found. Yet Anderson noted that the pancreatic ducts had been dissected infrequently and that an obstruction of the pancreatic ducts was observed in only a few cases. In 4 of her cases it was impossible to dissect the duct, but in only one of her protocols (no. 20) was definite atresia described; in 2 cases recorded in the literature stenosis of the duct was found near the orifice, and Anderson concluded that in 3 other instances definite atresia was observed. This last figure should be reduced to 1. She listed the reports by Tiling [2] and Benoit [3] as reports of 2 separate cases; yet they are descriptions of the same case. In the case reported by Siwe,[4] which Anderson lists as one of atresia of Wirsung's duct, there was a normal patent duct extending from the head to the tail of the pancreas, and Siwe considered the pancreatic lesion as due to "agenesis" of the excretory acinar tissue.

In all, therefore, there are only 4 cases recorded in the literature in which an obstructed pancreatic duct was seen to cause cystic fibrosis of the pancreas in infancy. A brief summary of the data on these cases follows:

Kornblith and Otani [5] reported the condition in a 5 day old infant. With serial microscopic sections they saw the main pancreatic duct, which was dilated throughout the body of the pancreas, suddenly narrow to a slitlike lumen in the head of the pancreas. Though the duct was stenotic, its lumen was not obliterated. Parmelee [6] noted in a 4½ year old girl that the duct at the head of the pancreas was "very narrow." Tiling [2] and Benoit [3] separately reported the condition in the same 7 month old infant. Actual atresia of Wirsung's duct was demonstrated in serial sections. The orifice of the duct was located in the duodenum beneath the common bile duct; the duct was then traced in the duodenal

1. Anderson, D. H.: Am. J. Dis. Child. **56**:344, 1938.
2. Tiling, W.: Arch. f. Kinderh. **106**:9, 1935.
3. Benoit, W.: Endokrinologie **16**:313, 1935.
4. Siwe, S. S.: Deutsches Arch. f. klin. Med. **173**:339, 1932.
5. Kornblith, B. A., and Otani, S.: Am. J. Path. **5**:249, 1929.
6. Parmelee, A. H.: Am. J. Dis. Child. **50**:1418, 1935.

wall and was seen to branch, but it immediately narrowed down to nothing and ended without ever penetrating through the muscularis of the duodenum or entering the pancreas. Finally Anderson [1] in the protocol of case 20, that of a 3 year old girl, noted that a small pancreatic duct was found which on gross examination extended from the ampulla for 8 mm. and was then "lost in fibrous tissue." No microscopic sections of this lesion were described.

Since the publication of Anderson's review, 2 other cases of pancreatic steatorrhea have appeared in the literature. In the case reported by Thomas and Schlutz [7] the pancreatic duct was dilated. The cause of the lesion was not found. In the case observed by Davie,[8] although Santorini's duct was patent, Wirsung's duct was thought to be occluded by fibrous tissue. Unfortunately, the pancreas was separated from the duodenum, and the orifice of the duct was not seen, nor was any histologic description of the duct recorded.

To make this summary of cases complete, I should like to add 5 cases of congenital cystic pancreatic fibrosis found by a review of the autopsy records of this hospital; these cases were noted briefly by Rich and Duff.[9] The precise obstruction was not demonstrated in any of these cases, but in 2 of them a suggestive microscopic lesion of the duct was seen, which had not been recognized grossly. In an autopsy on a 6½ month old boy the section of the ampulla of Vater showed a "multilocular system of glands suggesting a sort of papillomatous tumor growth" which might well have been the cause of the pancreatic lesion, although without serial sections this could not be established. Likewise, in an autopsy on a 3½ month old girl a section near the ampulla showed a large thick-walled pancreatic duct in which the mucosa was thrown up in papillomatous folds that narrowed the lumen, but in the absence of serial sections the nature of this lesion could not be determined.

From this survey it can be seen that a histologic picture of the lesion in the pancreatic duct causing cystic fibrosis of the organ is rare indeed. A case is reported, therefore, as in it serial sections were made of all the pertinent material. It seems advisable to add this case to the small number already described.

REPORT OF A CASE

A 10 month old white girl had always presented a feeding problem. She failed to gain well and vomited rather frequently. At 6 months she was admitted to the Harriet Lane Home with an infection of the upper respiratory tract and severe diarrhea. She was malnourished and dehydrated and had acute pharyngitis and otitis media. The diarrhea subsided in about two weeks. She ate well in the hospital, without vomiting, but did not gain well in spite of a large caloric intake. The stools were always large and dry, and an analysis of them showed poor absorption of fats. The dextrose tolerance was normal, but there was a tendency to alimentary glycosuria when the carbohydrate intake was large. There was a period of transient pyelitis, which was complicated by another attack of diarrhea. The baby had shown a tendency to wheeze, but no allergic reaction could be demonstrated. She was gaining fairly well when, nine days before her death, she

7. Thomas, J., and Schlutz, F. W.: Am. J. Dis. Child. **56**:336, 1938.

8. Davie, T. B.: J. Path. & Bact. **46**:473, 1938.

9. Rich, A. R., and Duff, G. L.: Bull. Johns Hopkins Hosp. **58**:212, 1936.

again acquired an infection of the upper respiratory tract, followed by bronchitis, bronchiolitis and pneumonia. The clinical impression was: pancreatic deficiency; vitamin A deficiency secondary to poor absorption of fats; diffuse bronchitis, bronchiolitis and pneumonia.

Pathologic Observations.—Autopsy was performed one and one-half hours after death. Only the important changes are noted here. The body weighed only 5.5 Kg. Nutrition was poor. There were no teeth. The abdomen was distended. The lungs showed foci of lobular consolidation, and pus-filled bronchioles were conspicuous. The liver was large and contained a good deal of fat. The gallbladder and bile ducts and the other viscera were normal. The lines of ossification of the bones were all straight and narrow.

The pancreas was the center of interest. It was left connected to the duodenum. It seemed to be of normal size and shape. No orifice corresponding to that of Santorini's duct was found in the duodenum. A minute orifice, thought to belong to Wirsung's duct, was seen adjacent to the ampulla of Vater. This was so narrow that a probe could not be passed. For this reason the pancreas was sectioned, but its ducts could not be seen, nor were any dilated channels or cysts noted at this time. In fact, the lobulation seemed about normal. However, a few hours after fixing the specimen in Zenker's fluid a striking change was seen. Instead of showing normal lobulation, the surface was covered with white foci varying in size from minute dots to spots with a diameter of 2.5 mm. The largest foci were easily seen to be cavities filled with inspissated material. When any of these cysts was probed it was found to end blindly, and there was no area that could be identified as a duct.

Microscopic Observations.—Only the abnormalities are described.

(*a*) Respiratory Tract: The mucosa of the trachea showed in places a thick layer of squamous epithelium replacing its normal columnar cells. Squamous epithelium even lined many of the ducts and acini of the mucous glands and was found in the main bronchi and also in tiny bronchioles in the depth of the lungs. The bronchioles were filled with an exudate of polymorphonuclear leukocytes. Their walls were thickened by infiltrating leukocytes and wandering cells. A few patches of peribronchial alveoli in both lungs were filled with purulent exudate.

(*b*) Rib: The line of ossification of the rib was entirely normal.

(*c*) Genitourinary Tract: The renal parenchyma was normal. The pelvic mucosa was greatly thickened, but the cells maintained their transitional character. The ureteral mucosa was also thick, but it too was normal transitional epithelium. The epithelium of the bladder formed a thicker layer than normal but showed no metaplasia. Thick squamous epithelium lined the vagina and cervix but did not extend into the uterus.

(*d*) Gastrointestinal Tract: The mucosa of the ducts of the salivary glands was appreciably thickened, and in some places areas of squamous metaplasia were seen. No ducts were occluded, but some dilated acini were seen. The epithelium of the esophagus formed a much thicker layer than normal. Patches of mononuclear cells were seen in its submucosa. In frozen sections of the liver stained with scarlet red fine globules of fat were seen in every cell.

(*e*) Pancreas and Duodenum: Serial sections showed the minute orifice identified grossly as the orifice of Wirsung's duct. The conclusion that this did not extend into the pancreas was substantiated, as microscopically (fig. 1) it

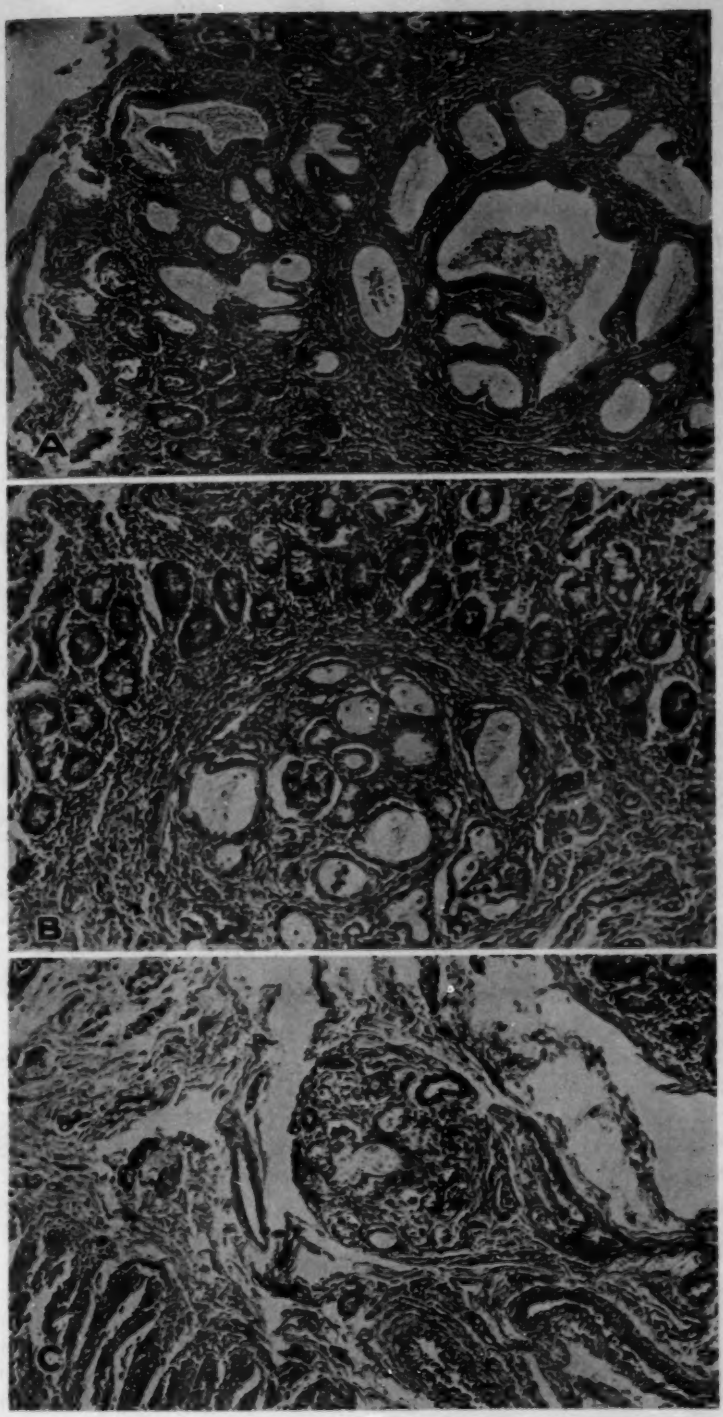

Fig. 1.—*A*, photomicrograph of a section of the orifice of Wirsung's duct as it enters the mucosa of the duodenum; × 100. *B*, Wirsung's duct in the submucosa, already greatly narrowed; × 100. *C*, Wirsung's duct just before its obliteration in the connective tissue of the submucosa; × 100. The duodenal mucosa is just visible in the right upper corner, and the muscularis of the duodenum is seen in the lower left corner.

consisted of a series of multilocular cysts which communicated and opened into the lumen of the duodenum but did not penetrate in the opposite direction through the muscularis of the duodenum. The lining epithelium was flattened, but there was no squamous metaplasia. Serial sections of the pancreas showed complete alteration of its structure (fig. 2). It consisted of a series of large and small cysts containing a coagulum which in places was inspissated and even calcified. The cysts were embedded in a fibrous stroma. All of the cysts could be traced in the serial sections until they disappeared, and none extended through the tissue as would a duct; nor could any continuous lumen resembling a duct be found. The ducts and acini were so dilated as to be indistinguishable. No squamous metaplasia was found in the pancreatic tissue. Numerous islands of Langerhans of normal structure were seen in the fibrous stroma.

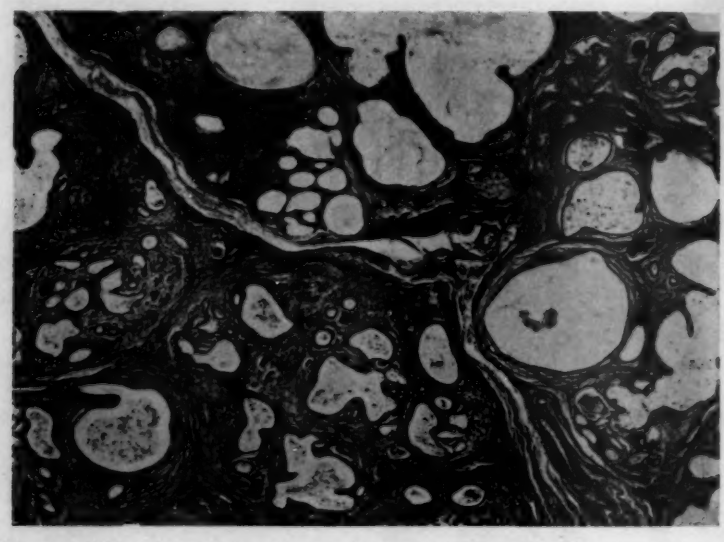

Fig. 2.—Photomicrograph of a section of pancreas showing irregular cystic dilatations embedded in dense connective tissue; × 40. Some of the cysts contain coagulum.

Final Anatomic Diagnosis.—Congenital atresia of the main pancreatic duct; cystic atrophy of the pancreas; vitamin A deficiency; squamous metaplasia of the trachea, bronchioles and salivary glands; purulent bronchitis; lobular pneumonia.

COMMENT

In this case of cystic fibrosis of the pancreas in a 10 month old baby, serial sections revealed the pancreatic lesion to be caused by true atresia of the pancreatic duct. This case is similar to that described by Tiling and Benoit. The pancreatic duct was lost in fibrous tissue before it penetrated through the muscularis of the duodenum. In the pancreas only dilated cysts, representing ducts and acini, were found, and none was connected to the duodenum. Fairly normal epithelium lined these cysts, so that this change could not be caused by occlusion of the pan-

creatic duct by squamous metaplasia. Quite the reverse had occurred. As the pancreatic duct was occluded, there were no pancreatic ferments in the duodenum, with resulting poor absorption of fats and a characteristic fatty condition of the liver. Anderson also noted this hepatic change in many of her cases. Furthermore, the poor absorption of fats had evidently been the basis for the vitamin A deficiency, i. e. the squamous metaplasia in the epithelium of the respiratory tract. Anderson also found this present in her cases and quoted Basu's earlier observation [10] that there is inadequate absorption of vitamin A from the alimentary tract if absorption of fat is inadequate. It is interesting that the bones of this child showed no rachitic change. In Anderson's cases, although vitamin A deficiency was a frequent finding, rickets was present only once. This indicates clearly that vitamin A is absorbed only with the fats absorbed from the intestinal tract, and in the absence of this source of the vitamin no vitamin A is acquired, whereas in the absence of intestinal intake of vitamin D a substituting factor, such as sunlight, may provide the vitamin to the body.

Blackfan and Wolbach [11] described terminal bronchitis and pneumonia secondary to the squamous metaplasia of the epithelium of the respiratory tract, which occurs in infants suffering from vitamin A deficiency. Anderson noted that this was invariably the cause of death in her patients. In this infant, too, purulent bronchitis, bronchiolitis and pneumonia were the cause of death.

SUMMARY

Cystic fibrosis of the pancreas in an infant is reported. Serial microscopic sections showed that congenital atresia of the main pancreatic duct was the basis of the pancreatic lesion. Poor absorption of fats and deficiency of vitamin A resulted, and death occurred from pulmonary infection secondary to squamous metaplasia of the bronchial epithelium.

10. Basu, N. K.: Ztschr. f. Vitaminforsch. **6**:106, 1937.
11. Blackfan, K. D., and Wolbach, S. B.: J. Pediat. **3**:679, 1933.

SPONTANEOUS DOUBLE RUPTURE OF THE HEART

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Spontaneous rupture of the heart is not rare.[1] It has been recorded in about 0.0016 per cent of necropsies and is estimated [2] to occur in about 2 to 6 per cent of myocardial infarctions. The anterior wall of the left ventricle is the commonest site of rupture. The interventricular septum is rarely involved. I have been unable to find a report of any case of septal rupture subsequent to the 26 cases mentioned in the survey by Benson, Hunter and Manlove,[1c] in 1933. These and other reports [3] make an example of double rupture of the heart seem unique.

REPORT OF A CASE

A man thought to be about 60 years old, with a history of old hemiplegia, convulsive seizures and inability to talk, was admitted to Western State Hospital, Dec. 17, 1931. He was found to have spastic right hemiplegia, motor aphasia and "irregularity in the rhythm and force of the heart tones." The clinical diagnosis was "psychosis with organic brain disease (cerebral hemorrhage)." While in the hospital, he gradually deteriorated mentally and physically and had occasional convulsive seizures with transient cyanosis and irregularities of pulse. Feb. 11, 1939, he was found to have edema of the legs. He suddenly became dyspneic and cyanotic, with a weak pulse. Two days later he was found dead, fifteen minutes after having "seemed as usual."

Necropsy revealed an obese white man 176 cm. in length, weighing about 210 pounds (95 Kg.), with dependent edema. The tense bluish pericardial sac was of triangular outline and measured 17.5 cm. transversely. Within it were about 200 cc. of fluid blood and a soft red clot weighing 476 Gm. The greatest trans-

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1. (*a*) Krumbhaar, E. B., and Crowell, C.: Am. J. M. Sc. **170**:828, 1925. (*b*) de la Chapelle, C. E.: Am. Heart J. **1**:315, 1926. (*c*) Benson, R. L.; Hunter, W. C., and Manlove, C. H.: Am. J. Path. **9**:295, 1933. (*d*) Salzmann, H. A.: Am. J. M. Sc. **188**:347, 1934. (*e*) Peterson, M. C.: Minnesota Med. **17**:256, 1934. (*f*) Clowe, G. M.; Kellert, E., and Gorham, L. W.: Am. Heart J. **9**:324, 1934. (*g*) McNamara, F. P.; Hancock, J. C., and Coady, C. C.: J. Iowa M. Soc. **27**:210, 1937.

2. The Criteria Committee of the New York Heart Association: Nomenclature and Criteria for Diagnosis of Diseases of the Heart, New York, J. J. Little and Ives Co., 1939.

3. Davenport, A. B.: Am. J. M. Sc. **176**:62, 1928. Stevenson, R. R., and Turner, W. J.: Bull. Johns Hopkins Hosp. **57**:235, 1935. Carter, D. Y.: Brit. M. J. **2**:335, 1936. McNamara, W. L.; Ducey, E. F., and Baker, L. A.: Am. Heart J. **13**:108, 1937.

verse diameter of the anterior surface of the heart was 14 cm. There was extensive petechial and ecchymotic hemorrhage beneath the epicardium of all cardiac chambers posteriorly. The right ventricle was empty. The right auricular appendage was distended by adherent grayish thrombi; the left one was empty and contracted. On the posterior surface of the left ventricle, 1.5 cm. to the left of the posterior interventricular sulcus and 3 cm. from the atrioventricular sulcus (*A* in figure), was a ragged longitudinal laceration 2.2 cm. long. The myocardium at its edges was soft, friable and yellowish. The heart weighed 570 Gm. The right coronary artery was considerably larger than the left, and its lumen was

A, posterior view of the septal areas of the ventricular wall showing the rupture through the posterior wall of the left ventricle. The bristle passes through the septal rupture. *B*, anterior view of a section through the septum and ventricles. The long bristle passes through the septal rupture which connects the two ventricles. The short bristle passes from the hemorrhagic cavity in the septum into the left ventricle. The internal opening of the rupture in the posterior left ventricular wall is just above the level of the long bristle, between the left edge of the septum and the knife cut which is visible in the posterior left ventricular wall.

unevenly distorted and narrowed by yellowish atheromatous plaques. Beginning at a point 15 mm. from the right coronary ostium, the vessel was occluded for a distance of 20 mm. by a dry, grayish and reddish thrombus. The artery passed

down the posterior interventricular sulcus as the posterior interventricular artery. The lumen of the left coronary artery, although tortuous and narrowed by atheromatous plaques with focal calcification, was patent throughout. Its circumflex branch turned downward and terminated over the posterior surface of the left ventricle just to the right of the obtuse margin without demonstrable anastomosis with the right coronary artery. The foramen ovale, though anatomically patent, was guarded by valvelike flaps adequate to close it. Both ventricles were dilated, and their papillary muscles and trabeculae carneae were thickened and flattened. The anterior wall of the right ventricle averaged 5 mm. in thickness; the posterior wall was about 7 mm. thick. Posteriorly, the muscular interventricular septum bulged into the right ventricle. The tricuspid valve was normal except for petechial hemorrhages on its posterior cusp, and the pulmonic valve was unchanged. The anterior wall of the left ventricle was elongated and measured 15 to 20 mm. in thickness, while the posterior wall was 9 to 12 mm. thick. Except for atheromatous plaques on the aortic cusps and on the anterior mitral leaflet, these valves were normal. There was extensive subendocardial hemorrhage of both left papillary muscles, over the posterolateral portions of the left ventricular wall and on both sides of the interventricular septum. The myocardium of the posterior wall of the left ventricle, the posterior papillary muscle, the upper posterior part of the septum and the posterior wall of the right ventricle were friable, softened, swollen, opaque and mottled reddish yellow and displayed tiny fibrotic foci and edematous areas. Elsewhere the muscle was coarsened, pinkish and firm. In the upper posterior part of the muscular septum (*B* in the figure) was a ragged tear, through which the ventricles communicated. The edges of this were covered by an adherent grayish red clot. On the right side, the septal rent measured 13 by 3 mm.; on the left side it measured 12 by 8 mm. The opening sloped downward to the right and slightly forward. There was hemorrhagic dissection anteriorly into the septum in a plane parallel to the muscle fibers, and the cavity thus formed communicated with the opening into the left ventricle. The internal dimensions of the rupture in the posterior wall of the left ventricle were 10 by 4 mm. About this opening was a reddish clot.

Microscopic examination showed slight adventitial lymphocytic infiltration of the right coronary artery with marked atherosclerotic stenosis and occlusion of the lumen by a recently formed thrombus, in which there was variably complete disintegration of the blood cells but no evidence of organization. The thrombi in the right auricular appendage showed softening but no organization. Here some of the myocardial cells were swollen, and there was patchy interstitial edema with infiltration by leukocytes, chiefly polymorphonuclears. The myocardium of the anterior wall of the right ventricle was hyperemic and edematous, and there was leukocytic exudate in the subepicardial fat. The anterior wall of the left ventricle and the anterior papillary muscle were modified by focal interstitial edema, slight, patchy fibrosis, focal infiltration by leukocytes and exudative inflammation of the subepicardial fat. The posterior right ventricular wall showed patches of young connective tissue containing lymphocytes and histiocytes and some necrobiotic changes in the muscle. The myocardium of the posterior left ventricular wall and of the corresponding papillary muscle exhibited focal recent fibrosis, edema, extensive coagulative necrosis, fresh hemorrhage and infiltration by well preserved leukocytes. About the septal rupture the muscle had undergone extensive coagulative necrosis and softening, with edema, hemorrhage and marked disintegration of the leukocytes and red cells of the abundant exudate. Here also were areas of vascular young granulation tissue containing old blood pigment, some of which was phagocytosed by histiocytes. All sections showed marked hypertrophy of the

muscle cells, which was greatest in those from the left ventricle. In some of the sections small intramural branches of the coronary arteries showed slight intimal fibrosis and atheromatous change.

Other important pathologic changes were: marked generalized arteriosclerosis with multiple ulceration and mural thrombosis of the aorta; cerebral arteriosclerosis with multiple foci of cerebral, pontile and medullary softening; general chronic passive hyperemia with ascites; anasarca and bilateral hydrothorax with compression atelectasis of the congested and edematous lungs; bilateral renal arteriosclerosis without nephrosclerosis; nodular fibroadenomatous hyperplasia of the prostate gland, and purulent ethmoiditis and sphenoiditis. The thyroid and other endocrine glands were normal.

COMMENT

Although not of clinical record, the presence of arterial hypertension may be presumed because of the renal arteriosclerosis and the marked hypertrophy of the heart, predominantly left ventricular, without other demonstrable cause. This hypertension was no doubt an important cause of the two distinct ruptures through the same myocardial infarct. It would seem that the septal rupture probably occurred first, possibly two days before death, when the symptoms of acute cardiac distress appeared so abruptly in this person with failing circulation. The septal portion of the infarct was the older of the two foci, since a thrombus had formed over it. The massive tamponading intrapericardial hemorrhage would have been fatal before thrombosis could have occurred had the ruptures been simultaneous or had the mural rhexis occurred first. If this assumption is true, the present case is additionally interesting for the reason that solitary septal ruptures usually kill as suddenly as do mural ones. Anastomosis between the anterior and posterior interventricular arteries apparently spared the anterior apical septum from infarction, but the circumflex branch of the left coronary artery was apparently incapable of saving much of the left ventricle.

SUMMARY

A unique example of thrombotic occlusion of the right coronary artery with extensive myocardial infarction and rupture through the posterior part of the muscular interventricular septum, with subsequent separate rupture through the posterior wall of the left ventricle, resulting in fatal hemopericardium, is reported.

ENDOMETRIAL SARCOMA

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Endometrial sarcoma has been recognized as a pathologic identity for a long time. In fact, Virchow [1] described the diffuse type of this neoplasm in 1865 and agreed that polypoid types might occur. During the latter part of the nineteenth and the early part of the twentieth century, numerous cases were reported. Many were included in compilations of cases of sarcoma of the uterus and were not published under the title of endometrial sarcoma. References to this older literature may be found in Piquand's [2] review of 1905 and in Ewing's [3] "Neoplastic Diseases."

Despite the voluminous early literature, endometrial sarcoma is today a little known entity. Stout,[4] for instance, stated that he has seen only 1 case. He believes that in many of the older reports cases of undifferentiated carcinoma were included among the cases of sarcoma.

Masson [5] compiled a series of 50 cases of uterine sarcoma that were observed during a period of twenty years and found only 1 case of endometrial sarcoma.

The difficulty in diagnosis may be responsible for the apparent rarity, with the result that cases of endometrial sarcoma are classified as cases of leiomyosarcoma. On the other hand, the frequency of reports of cases of endometrial sarcoma in the older literature may be due, as Ewing [3] said, to the fact that the cases were instances of advanced mural leiomyosarcoma.

R. Meyer [6] followed the teachings of Virchow and on the basis of gross anatomic features distinguished between a typical diffuse type of endometrial sarcoma and a polypoid type. He described the diffuse type as closely resembling histologically the lamina propria of the endometrium and, like it, possessing a typical reticulum, consisting of delicate fibrils surrounding individual cells. On the other hand, the polypoid forms are more akin to the mucosal polyps than to the lamina propria of the endometrium, possessing as they do a fibrillary stroma and spindle cells. Atypical and mixed cells are found in the latter type.

From the Laboratory of Pathology, Westfield State Sanatorium.

1. Virchow, R.: Die krankhafte Geschwülste, Berlin, A. Hirschwald, 1864-1865, vol. 2 (*a*) p. 350; (*b*) p. 180.

2. Piquand, G.: Rev. de gynéc. et de chir. abd. **9**:387, 1905.

3. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1929, p. 284.

4. Stout, A. P.: Human Cancer, Philadelphia, Lea & Febiger, 1932, p. 357.

5. Masson, J. D.: Am. J. Obst. & Gynec. **5**:345, 1923.

6. Meyer, R., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 7, pt. 1, p. 374.

Since 1918, scattered reports have appeared in the literature under the title of endometrial sarcoma. Brady,[7] Gerich,[8] Ward,[9] Offergeld,[10] Moench and Meeker[11] and Burger[12] have each published 1 case. Casler[13] described a "unique, diffuse, uterine tumor really an adenomyoma with stroma but no glands" in a woman 39 years of age. This was no doubt an endometrial sarcoma arising from the lamina propria of the endometrium.

Tudhope and Chisholm[14] described 3 cases which they thought might be mistaken for cases of endometrial sarcoma. Close examination of tissues routinely stained, as well as of those stained by Foot's modification of Bielschowsky's silver stain for reticulum, revealed, according to the authors, that all were instances of undifferentiated carcinoma.

My own experience has led me to believe that this neoplasm is not well known among pathologists and gynecologists. Certainly few reports have appeared in the literature recently. It is with these points in mind that the following 2 cases are reported.

REPORT OF CASES

CASE 1.—A white woman 65 years old entered the cancer section of the Westfield State Sanatorium, Dec. 9, 1937, because of intermittent vaginal bleeding since November 1937. The menopause had occurred at the age of 46. There was a spherical uterus, about 8 cm in diameter, with a fungating growth within it. On December 17, after dilation and curettage, 50 mg. of radium was inserted into the body of the uterus; a total dose of 2,400 milligram hours was given. The pathologic diagnosis on the basis of the curettings was endometrial sarcoma. External irradiation was attempted but discontinued after the patient had received 600 roentgens to the pelvis on account of low red cell counts. Three transfusions were given, and a total hysterectomy was done.

The surgically removed specimen was a uterus amputated above the cervix, with tubes and ovaries attached. The cervix was submitted separately. The uterus measured 6 by 6.5 by 6 cm. The serosa was smooth. When the anterior wall was sectioned, a large, soft, yellowish brown, broad, lobulated polypoid mass was found attached to the posterior wall by a broad base, completely filling the endometrial cavity. It measured 8.5 by 9.5 by 2.5 cm., having expanded when the uterus was opened. On section it showed yellowish brown tissue with the consistency and appearance of cerebral tissue. Numerous regions of cystic degeneration were present. There were hemorrhagic foci on the surface. There was little invasion of the myometrium at the base on gross examination. The myometrium throughout the uterus, both at the base of the neoplasm and elsewhere, measured 1.5 cm. in thickness. The endometrium covering the anterior wall was smooth and thin. A small leiomyoma, measuring 0.8 cm., was found just above the cervix in the anterior wall, and another of the same size, in the left cornu. Both the

7. Brady, L.: Bull Johns Hopkins Hosp. **29**:164, 1918.
8. Gerich, O.: Zentralbl. f. Gynäk. **53**:2016, 1929.
9. Ward, C. V.: Canad. M. A. J. **25**:707, 1931.
10. Offergeld, H.: Ztschr. f. Krebsforsch. **39**:191, 1933.
11. Moench, G. H., and Meeker, L. H.: Am. J. Obst. & Gynec. **30**:435, 1935.
12. Burger, P.: Bull. Soc. d'obst. et de gynéc. **25**:274, 1936.
13. Casler, DeW. B.: Surg., Gynec. & Obst. **31**:150, 1920.
14. Tudhope, G. R., and Chisholm, A. E.: J. Obst. & Gynaec. Brit. Emp. **41**: 708, 1934.

ovaries and the tubes were normal. The cervix measured 3.5 by 2.0 by 2.5 cm. A patulous orifice with bilateral tears was present.

Section through the neoplasm revealed replacement of the endometrium by cellular, compact, homogeneous neoplastic tissue resembling to a striking degree the lamina propria of endometrium. No acini were visible. The neoplastic cells possessed small oval to round nuclei, containing fine chromatin granules. There was an occasional small nucleolus. There was little variation in the size and shape of the cells. There was scanty cytoplasm with indistinct cell outlines. A small amount of intercellular substance was present. There were numerous small arteries and veins with hyperplasia of the endothelium and mural cells. Numerous mitotic figures were seen. There was superficial, but definite, invasion of the myometrium. An intact layer of simple columnar epithelium was seen on the surface. No effect of irradiation was noted. With Wilder's reticulum stain, delicate reticular fibers formed a mesh resembling closely that found in the lamina propria of the endometrium. Reticular fibers were in intimate relationship with each cell, curving about and practically enclosing each one individually. These reticular fibers were much thinner than those in the myometrium.

The rest of the endometrium was atrophic. Small, ill defined nodules of criss-crossing bundles of smooth muscle formed small leiomyomas within the myometrium. There was moderate lymphocytic infiltration of the cervical stroma, with few dilated glands visible. The ovaries and tubes were normal.

The diagnosis was endometrial sarcoma, leiomyoma of the uterine fundus and chronic cervicitis.

The patient died Jan. 29, 1938, with signs of cardiac failure.

Laboratory Data.—Dec. 10, 1937, the red cell count was 1,420,000; the hemoglobin content 2.2 Gm. (Sahli); the white cell count, 5,900. Jan. 22, 1938, the red cell count was 3,850,000; the hemoglobin, 10.2 Gm. (Sahli); the white cell count, 8,400.

Postmortem Examination.—The body was that of an elderly white woman, slightly emaciated. The abdomen was distended. A recent abdominal incision, 17 cm. in length, held together by stay sutures, extended from a point at the left of the umbilicus to just above the pubis. The edges adhered well. A drain was present in the lower portion. The pelvis contained 25 cc. of fresh blood. The duodenum and the loops of the jejunum were distended. There was slight distention of the cecum. A defect in the pelvic floor caused by removal of the internal genitalia was well repaired, with sutures intact.

The heart weighed 430 Gm. There was dilatation of the right ventricle and right auricle. The left ventricle was moderately enlarged and the left ventricular wall thickened. On section, the myocardium of the left ventricle displayed a few yellowish white foci of fibrosis, measuring up to 0.5 cm. in diameter. There was a slight amount of fibrosis in the rings of the aortic and pulmonic valves.

The left lung weighed 300, the right 420, Gm. There was congestion of the posterior portions of the upper lobes, with atelectasis of the posterior portions of both lower lobes. The bronchi contained thick mucopurulent material. The main branches of the pulmonary artery were dilated; the secondary branches, within the lungs, were normal.

The left kidney weighed 240 Gm.; the right, 228. The capsules stripped with difficulty, exposing a somewhat irregular, reddish brown surface with scattered thin-walled cysts, measuring from 0.7 to 5.5 cm. in diameter. On section, the cortices were found markedly and irregularly narrowed, measuring 0.2 to 0.4 cm. in thickness. The demarcation between the cortex and the medulla was poor on the right and on the left. An encroachment of cysts on the medulla was visible.

The thyroid weighed 45 Gm. The left lobe contained a few small nodules measuring up to 3.5 cm. in diameter. The nodules were well encapsulated, translucent, yellowish. In the right lobe were similar but smaller nodules, measuring up to 1 cm. in diameter.

The left sacroiliac joint and vertebrae were examined, but no metastatic tissue was found.

The head was not examined.

Microscopic Examination.—Sections were stained with phloxine–methylene blue. In the heart were small patches of fibrosis and thickening of the intima of small arteries; in the lungs, marked congestion and hemorrhage. The kidneys showed numerous wedge-shaped cortical scars with scattered lymphocytes, many cortical and medullary cysts with thin, fibrous walls, lined with flattened epithelial cells, and thickening of the intima of medium-sized arteries. An occasional hyalinized arteriole could be seen. There was lymphocytic infiltration of the submucosa of the pelvis.

The aorta showed fibrous thickening of the intima with cholesterol deposits.

There was marked fibrosis in the nodules in the thyroid, with dilated acini, filled with colloid. There was much colloid in the connective tissue between the acini.

Bone from the iliosacral joint and vertebrae was normal. No metastases were observed.

The main postmortem diagnoses were panhysterectomy for sarcoma of the endometrium, hypertensive cardiac hypertrophy and paralytic ileus.

CASE 2.—A white woman 72 years old was admitted to the cancer section of the Westfield State Sanatorium Oct. 4, 1938. She had had vaginal bleeding since June 1937. There was a foul vaginal discharge. There had been dull aching pain in both lower quadrants of the abdomen for the past two months; also, slight frequency of urination. The loss of weight was 8 pounds (3.6 Kg.). The menopause occurred at the age of 55.

Physical examination revealed a large, hard tumor in the lower quadrant of the abdomen, reaching the umbilicus. A hard, nodular, freely bleeding mass was felt in the cervical canal. The uterus was freely movable.

October 7, after dilatation and curettage, a specimen was taken from the cervix for biopsy, and 100 mg. of radium in a platinum bomb was inserted. The patient received 1,700 milligram-hours of radium exposure. The pathologic diagnosis on the curettings was chronic cervicitis with necrosis of tissue. The patient was discharged against advice October 17 but was admitted a second time Jan. 20, 1939. Vaginal bleeding had continued. The pelvic condition was similar to that on first examination. Exploratory laparotomy was done January 30. A large uterus was found with an irregular nodule on the superior surface of the fundus, with two loops of small intestine attached. On account of inoperability, biopsy alone was done. The pathologic diagnosis on the tissue removed was "sarcoma, insufficient for classification." The patient died February 8.

Laboratory Data.—Jan. 5, 1938, the red cell count was 4,330,000; the hemoglobin content, 11.3 Gm. (Sahli); the white cell count, 6,300. February the red cell count was 3,300,000; the hemoglobin, 9.9 Gm. (Sahli); the white cell count, 20,200.

Postmortem Examination.—The body was that of an obese white elderly woman. The abdomen was markedly distended. A recent, poorly healed surgical incision extended from just above and to the right of the umbilicus down toward the suprapubic region for about 20 cm. There was an abundant amount of foul-smelling grayish purulent material in the vaginal orifice. The mucosa of the lower portion of the vagina was ulcerated and grayish green. The large and small intestines

were distended. Loops of small intestines were adherent with friable grayish fibrinous adhesions. About 600 cc. of a grayish brown purulent fluid was distributed generally. The enlarged uterus protruded from the pelvic cavity with a grayish black, mottled nodule on the superior aspect of the fundus. Two loops of proximal ileum were adherent to the nodule. The peritoneum was thickened and discolored grayish green to grayish black, especially on the right. The perineal fat was also thickened. There were abundant fibrinous adhesions between the right lobe of the liver and the right dome of the diaphragm. The sigmoid was compressed by the enlarged uterus and was adherent to the left lateral gutter by means of dense fibrous adhesions. The pouch of Douglas was obliterated. The omentum was moderately contracted, thickened and indurated, with an abundant amount of fat present. It revealed indistinct yellowish white striations.

In the pleural cavities there were dense fibrous adhesions at the apexes of the left and right lungs. On the right 50 cc. of straw-colored fluid was present. There were scattered yellowish plaques with slight calcification in the descending branch of the left coronary artery just below its junction with the left circumflex artery. In the lower lobe of the left lung there were superficial regions of atelectasis. On the right, atelectasis was more extensive, with half of the lower lobe involved.

The gastrointestinal tract was normal, but there was moderate induration of the perirectal tissues by fibrosis, with a small yellowish white nodule, about 0.7 cm. in diameter, in the anterior portion of the perirectal tissues. The distal portion of the gallbladder was fibrotic and narrowed and enclosed two pigment-cholesterol stones. There were small pigment stones in the neck. The common duct was dilated, 1.4 cm. in diameter. The kidneys each weighed 160 Gm. The cortices were well demarcated from the medullae but were narrowed, measuring 0.3 to 0.4 cm. in thickness. There was a small cyst with purplish fluid in the upper pole of the left kidney. In the bladder, in the region of the trigon, were small, slightly translucent, reddish brown excrescences, 0.1 cm. in diameter.

The fundus of the uterus was much enlarged, measuring 13 by 9.5 by 9.5 cm. The cervix measured 1.5 cm. in length. The upper portion of the anterior surface of the fundus was smooth; the lower portion was adherent to the bladder by fibrous and fibrinous adhesions. The anterior wall of the uterus was sectioned, revealing a dilated endometrial cavity, measuring 18 cm. in circumference. It was filled with large, necrotic, friable, yellowish red to yellowish white polypoid masses with grayish green to grayish black tips. The entire endometrial surface was involved by the neoplasm except for the lower segment, measuring 3.5 cm. in length from the lower edge of the neoplasm to the internal cervical orifice. On section the neoplastic tissue possessed a variegated color pattern corresponding to the colors of the surface. Grossly, the uterine wall appeared more compressed than invaded, with only a thin rim of myometrium present, measuring from 0.2 to 1.5 cm. in thickness. The uterine wall of the superior portion of the fundus, where the nodule was found attached to loops of proximal ileum, was grayish green, necrotic and very thin, measuring about 0.2 cm. in thickness. The exocervix was flattened. The cervix itself was uninvolved by the neoplasm. The pericervical tissues were indurated and fibrotic. The entire vagina was ulcerated and grayish green. There was infiltration of the left broad ligament by the neoplasm; there was thickening of the right broad ligament, with no neoplastic infiltration. The left fallopian tube was slightly dilated, 0.7 cm. in diameter. The fimbriated end was obliterated and adherent to the cystic left ovary, which measured 1.5 cm. in diameter. There were flecks of fibrin on the surface of the left ovary. The right tube was more dilated, measuring 1.2 cm. in diameter. The surface was grayish green and covered with

flecks of fibrin. The fimbriated end was obliterated and adherent to the right ovary, which measured about 1.2 cm. in diameter.

The aorta showed scattered yellowish plaques, with early calcification present, especially in the lumbar region.

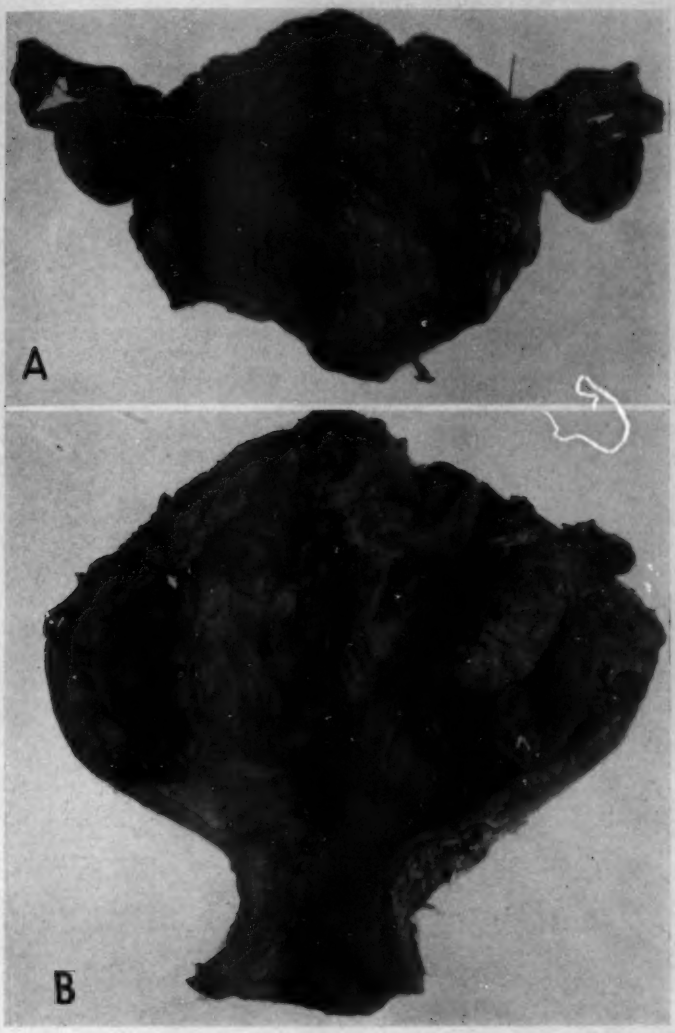

Fig. 1.—*A*, uterus with an endometrial sarcoma having its origin in the lamina propria (case 1). *B*, uterus with an endometrial sarcoma having its origin in an endometrial polyp (case 2).

The head was not examined.

Streptococcus haemolyticus (beta) was isolated from the peritoneal exudate after death.

EXPLANATION OF FIGURE 2

A, section of the endometrial sarcoma in case 1; hematoxylin-phloxine; × 430. Note the homogeneity of the cells and their similarity to those of the lamina propria of the endometrium.

B, delicate mesh of fibers similar to that found in the lamina propria of the endometrium enclosing the individual sarcomatous cells (case 1); Wilder's reticulum stain: × 430.

C, section of a rapidly growing, immature portion of the sarcomatous uterine neoplasm in case 2, showing a streaming bundle of characteristic sarcomatous spindle cells with mitotic figures; phloxine-methylene blue; × 430.

D, section of a slowly growing, mature portion of the uterine neoplasm in case 2, showing masses of carcinoma surrounded by sarcoma; phloxine-methylene blue; × 100.

E, section of a slowly growing, mature portion of the sarcomatous uterine neoplasm in case 2, showing coarse, slightly wavy long fibers running parallel to the long axes of sarcomatous cells; Wilder's reticulum stain; × 430.

F, section of a carcinomatous metastasis in perirectal tissues showing masses of carcinoma separated from sarcoma by a thin zone of acellular connective tissue (case 2); phloxine-methylene blue; × 100.

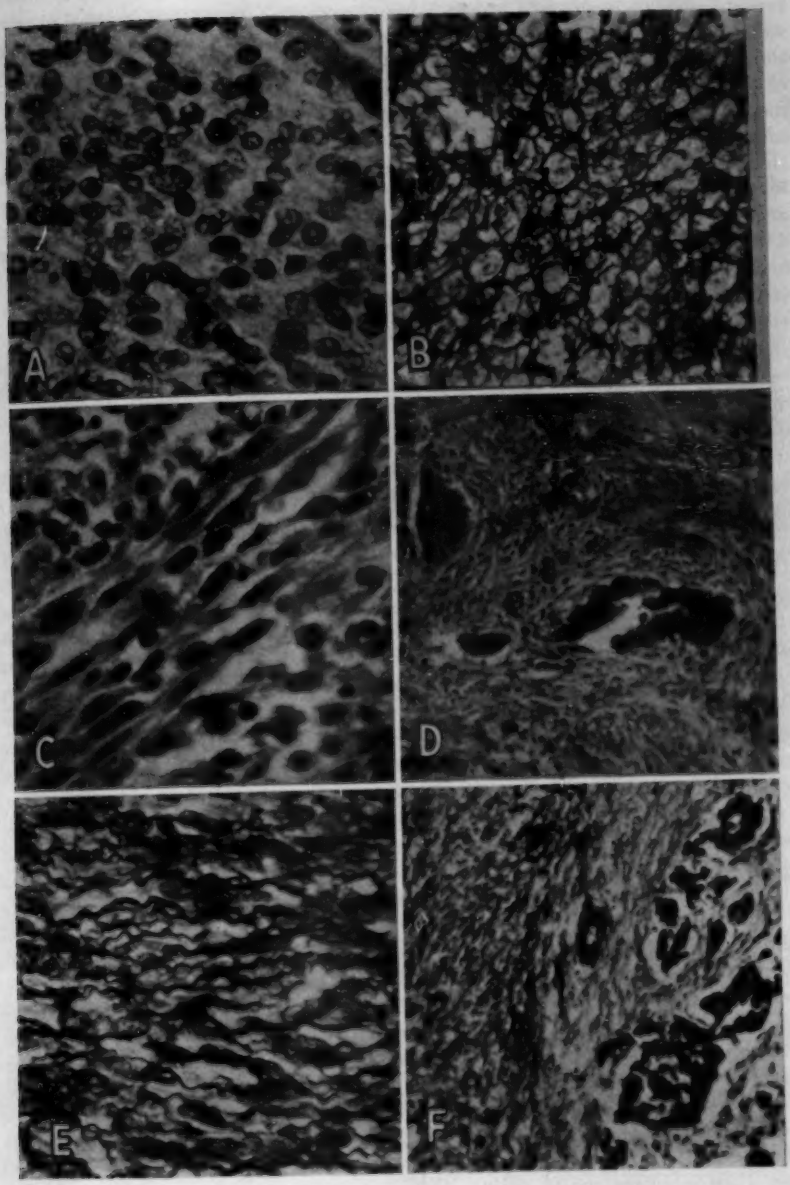

Figure 2

Microscopic Observations.—Sections were stained with phloxine–methylene blue, Masson's trichrome stain as modified by Goldner [15] and Wilder's stain for reticulum. The lungs and spleen were congested. There was marked edema, with deposition of fibrin, in the submucosa of the small intestine. There was thickening of the serosa by edema and fibroblastic proliferation with thick layers of fibrin and neutrophils on the surface. The rectum was normal, but small masses of compact, polygonal neoplastic epithelial cells were present within the perirectal tissues. The oval and round nuclei of the neoplastic cells contained coarse chromatic granules and occasional large nucleoli. There were scattered giant lobulated nuclei. An occasional mitotic figure was seen. The cytoplasm was acidophil and moderately abundant. There was infrequent acinus formation. There was edematous stromal connective tissue about the neoplastic epithelial masses, the former sharply differentiated from the latter. Thin stromal trabeculae separated the neoplastic epithelial masses from one another. The stroma was predominantly cellular; small vesicular spindle-shaped nuclei were present in most places, but no mitotic figures. There was little variation in size or shape. In one region, however, the stroma was definitely sarcomatous. Here, an acellular zone of connective tissue was present about the neoplastic epithelial masses, with sarcomatous tissue beyond. There was transition between the benign cellular connective tissue stroma and the sarcomatous tissue. The latter was arranged in criss-crossing bundles, with oval and spindle-shaped nuclei, which were definitely plumper and more hyperchromatic than those in non-neoplastic stroma. The nuclear chromatin material was arranged in fine granules, with occasional small nucleoli present. The cytoplasm was scanty. Numerous mitotic figures with atypical forms were present. There was moderate variation in size. The phloxine–methylene blue stain revealed a moderate amount of intercellular material. With Wilder's stain for reticulum, the masses of carcinoma were found to contain no silver-staining fibers, but the stromal reticulum about the carcinomatous masses was prominent. Within sarcomatous stromal portions the reticulum stood out sharply, with numerous thick and thin, long, slightly wavy, at times reduplicated silver-stained fibers noted in intimate contact with sarcomatous cells. This reticulum was similar to that found in more mature portions of the uterine neoplasm, to be described later.

A few small foci of fat-filled cells were noted in the liver. The wall of the gallbladder was thickened and fibrotic, with large foci of cholesterol and small foci of calcification. Scattered lymphocytes were noted. The serosa was edematous and infiltrated by neutrophils, lymphocytes and endothelial cells. One section of a kidney had a cyst between the cortex and the medulla, with a narrow zone of fibrous renal parenchyma at the periphery. There were wedge-shaped scars in the cortex. The lamina propria of the bladder showed moderate edema and congestion, with foci of lymphocytes scattered about. In one section there was invasion of the serosa by masses of neoplastic epithelial cells similar to those found in the perirectal tissue, but no sarcomatous tissue was present. Numerous sections through the uterine neoplasm revealed a predominantly rapidly growing, ulcerated, solid sarcomatous tissue arranged in criss-crossing bundles with a herringbone pattern visible. There were scattered thin-walled vessels. The cells were typically fibroblastic. The nuclei were predominantly spindle shaped with some oval, round and giant lobulated nuclei visible. They contained fine chromatin granules and round, prominent, single or multiple nucleoli. There were numerous mitotic figures. The cytoplasm was granular, collected at the poles and tapered to fine points.

15. Goldner, J.: Am. J. Path. **14**:237, 1938.

There was much necrosis. The nonulcerated portions were covered by simple columnar epithelium. There was marked invasion of myometrium, with isolated foci of muscle surrounded by sarcomatous tissue. There was a thin layer of myometrium at the periphery in some sections. There was invasion of veins by the neoplasm. There was organizing fibrin on the serosa. In this rapidly growing sarcomatous tissue, occasional coarse intercellular fibrils were visible with phloxine–methylene blue stain. With the trichrome stain, a few scattered intercellular collagen fibers were noted. Wilder's stain revealed a few coarse, long, slightly wavy reticular fibers running along the long axis of the cells.

In two sections the neoplasm was more mature and slowly growing. The surface was covered by simple columnar epithelium, occasionally indented, forming acini. Occasional small solid epithelial masses were also present. Within acini the epithelial cells were often arranged in several layers. The epithelial nuclei were oval to round with occasional definitely neoplastic, multinucleated and multilobular giant cells. The cytoplasm was scanty and acidophilic, with occasional round acidophilic cytoplasmic inclusion bodies present. Papillary projections within acini were composed of sarcomatous tissue covered by epithelial cells. The trichrome stain revealed much more intercellular collagen here than in rapidly growing portions. The tissue did not stain like smooth muscle. Wilder's stain for reticulum revealed numerous coarse, long, slightly wavy fibrils along the long axes of cells in intimate contact with them. These fibers were similar to those in the stromal sarcoma of the perirectal tissues. No irradiation effect was noted in the uterine neoplasm. There were numerous lymphocytes and polymorphonuclears within the mucosal folds and in the lumens of the tubes. In one section of ovary there were small carcinomatous masses at the periphery, similar to those in the perirectal tissues. The stroma about the carcinomatous masses was also similar to that in the perirectal tissues except for absence of sarcomatous change. A small focus of sarcoma was noted at some distance from the carcinomatous masses, with moderate polymorphism, similar to that seen in rapidly growing portions of the uterine sarcoma. The thyroid contained a small nodule composed of poorly developed acini. Many epithelial cells had vacuolated cytoplasm. There was marked necrosis of the surface of the abdominal wound with numerous neutrophils and lymphocytes below. No fibroblastic activity was seen. There were occasional foci of cellular marrow in an otherwise atrophic marrow in the vertebrae.

The main postmortem diagnoses were: endometrial sarcoma of the uterus with foci of carcinoma and local extension and metastases to the perirectal tissues, bladder and ovary; acute fibrinopurulent peritonitis.

COMMENT

The 2 cases described are offered as instances of the two types of endometrial sarcoma.

In the first the tumor, except for its uniradicular, polypoid appearance, was typical of sarcoma arising from the endometrial lamina propria. Consistent with this origin were the location of the neoplasm and the superficial invasion of the myometrium. In addition, not only did the characteristics of the cells stained with hematoxylin and phloxine leave no doubt of its histogenesis but the reticulum within the neoplasm was characteristic, simulating as it did the reticulum of the endometrial lamina propria. This reticulum was delicate and consisted of fibers which twisted and turned, enclosing each cell individually. This pattern was in sharp contrast to the long, thick reticular fibers in leiomyosar-

coma, which extend parallel to the long axes of the cells. Whether this type of endometrial sarcoma grows in an undifferentiated form and thus loses its reticulum could not be ascertained from the literature.

Although the importance of the reticulum in diagnosing this type of neoplasm has been stressed by R. Meyer,[6] none of the articles describing this type of neoplasm published within the past twenty years has mentioned the use of a silver reticulum stain. It is true that Tudhope and Chisholm [14] used a silver reticulum stain, but they were concerned with differentiating carcinoma from sarcoma, and furthermore they had no neoplasm similar to that in the first case reported here.

No metastases were found at postmortem examination, which was consistent with the histologic appearance.

The absence of any irradiation effect on this neoplasm is striking. The 600 roentgen of radiation may be disregarded, but the 2,400 milligram hours of radium exposure given over a month before the total hysterectomy must be taken into consideration despite the bulk of the neoplasm. It appears that, as in leiomyosarcoma, radical surgical operation is the only rational treatment.

The neoplasm in the second case, which is similar to one cited by Piquand,[2] falls into the category of the classic polypoid type of endometrial sarcoma arising from polypi. This is a logical classification, since the stroma of the endometrium gave rise to the stroma of the polyp which in turn gave rise to the sarcoma.

In the neoplasm described, elements related to those of a fibrous polyp were noted in the more slowly growing portions of the neoplasm, with carcinomatous glandular structures intermingled with the sarcomatous stroma. The sarcomatous cells, unlike those in the neoplasm of the first case, were more spindle in type.

The reticulum in this neoplasm resembled that in endometrial polypi, with long, thick fibers running parallel to the long axes of the cells. The reticulum alone is not of as much importance as in the first case, since a similar type can be seen in leiomyosarcoma, but it is valuable if used in conjunction with the general histologic picture.

Of course, the fibroblastic characteristics of the cells and the abundant amount of intercellular collagen in intimate relationship to the cells in the mature portions showed that this neoplasm was not leiomyosarcoma.

In addition, the sarcomatous elements of the uterine neoplasm were definitely not undifferentiated carcinoma, since the reticulum, especially that in the slowly growing portions, was abundant and in intimate contact with the cells. Furthermore, the reticulum separated sharply the carcinomatous from the sarcomatous elements. It would be contrary to all histologic criteria to believe that a carcinoma could produce such typical sarcomatous reticulum. The few reticular fibers in the rapidly growing portion were a sign of poor differentiation.

The carcinomatous elements formed histologically only a small part of the uterine neoplasm. In fact, they were absent in the rapidly growing portions. Clinically, they were important because they were capable of metastasizing.

Of great interest is the stroma of the carcinomatous metastases, since definite transformation of the stroma into sarcoma took place. It is

doubtful on histologic grounds that this change represented metastasis from the uterine sarcoma. Furthermore, simultaneous metastases of both the carcinomatous and the sarcomatous elements to an identical spot, with the latter surrounding the former, would be extraordinary. A third point against metastasis from the uterine sarcoma is the fact that transition from the benign to the sarcomatous stroma could be traced.

The last point is of interest, since it indicates an influence of the carcinomatous element on its stroma, which led to malignant changes. Of course, if this was so in the metastatic foci, it seems that the same might hold true for the primary uterine neoplasm, where the influence by the carcinomatous elements on the stroma of the original polyp might have given rise to the sarcoma which later became the predominant neoplasm.

It is of interest that this idea of a malignant neoplasm possibly influencing adjacent non-neoplastic tissue toward malignant neoplasia was mentioned by Virchow,[1b] who, however, expressed the opinion that the sarcoma preceded the carcinoma in cases similar to my second case. On the other hand, Ehrlich and Apolant,[16] who were the first to demonstrate such a phenomenon in animal neoplasms, were convinced that the carcinoma arose first and influenced its stroma to become sarcoma, which in turn became the predominant neoplasm. Herxheimer [17] and Harvey and Hamilton [18] agreed with Ehrlich and Apolant; they applied this idea to human neoplasms.

As in the uterine neoplasm, so in the metastatic foci, the sarcomatous elements could not be confused with undifferentiated carcinoma since a typical sarcomatous reticulum was found in the sarcomatous stroma, sharply differentiating them from the carcinomatous elements, which contained no reticulum.

No irradiation effect could be found on this neoplasm despite the application of 1,700 milligram-hours of radium exposure four months before death, although the bulk of the neoplasm, as in the first case, might have prevented easy access of the radium to the entire mass of the neoplasm. Radical surgical intervention would thus seem to be the best method of treatment for this type of neoplasm.

While I am aware that the second case might be regarded as an instance of carcinosarcoma, a discussion of this much debated subject (Saphir and Vass [19]) has been deliberately avoided to prevent this paper from becoming too diffuse and cumbersome.

SUMMARY

Of the 2 cases of endometrial sarcoma described, the first belongs to the group in which the neoplasm arises from the lamina propria of the endometrium, the second to the group in which it comes indirectly from the lamina propria by way of an endometrial polyp.

16. Ehrlich, P., and Apolant, H.: Centralbl. f. allg. Path. u. path. Anat. **17**: 513, 1906; Berl. klin. Wchnschr. **44**:1399, 1907.

17. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **44**:150, 1908; Centralbl. f. allg. Path. u. path. Anat. **29**:1, 1918.

18. Harvey, W. F., and Hamilton, T. D.: Edinburgh M. J. **42**:337, 1935.

19. Saphir, O., and Vass, A.: Am. J. Cancer **33**:331, 1938.

Within the predominantly sarcomatous uterine neoplasm of the second case were foci of carcinoma, which gave rise to metastases. The latter were surrounded by a cellular stroma which in one region was sarcomatous. This observation suggested that both the carcinoma in the uterus and that in the metastases might have influenced the stroma to become sarcoma, which in the uterus became the predominant neoplasm.

The value of reticulum staining to bring out the characteristic reticulum in endometrial sarcoma arising from the lamina propria has been stressed. The reticulum in the endometrial sarcoma arising from an endometrial polyp was found to be less characteristic. Reticulum staining was also of value in confirming the diagnosis of sarcoma in the stroma of the carcinomatous metastases of the second case and in distinguishing the sarcoma from undifferentiated carcinoma.

Laboratory Methods and Technical Notes

HAMDI'S PRESERVING SOLUTION

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My teacher, the late Prof. H. Hamdi, used an inexpensive fluid for the preservation of gross pathologic specimens, which has been in general use in our country for some years. The simplicity, low cost and efficacy of the method lead me to describe it here.

The specimen to be preserved is fixed in a 10 to 20 per cent dilution of the 40 per cent stock solution on the market. The larger and more solid the specimen (brain, liver, large spleen, large tumor) the higher the percentage of stock solution that may be used. After the specimen has been thoroughly fixed and prepared, it is left in running tap water for at least twenty-four hours; then it is placed in a highly hypertonic (about 50 per cent) salt solution prepared with clean tap water. Here it is left for two to five days. The hypertonic salt solution penetrates the organ, drives out the formaldehyde solution, causes a slight swelling, which removes the shrinkage due to the formaldehyde fixation, and increases the weight of the specimen, so that the lungs, for instance, do not float when placed in the preserving fluid. It helps, moreover, to prevent the growth of molds. After the organ has been rinsed in running water, it is placed directly into Hamdi's solution, the formula of which is as follows:

Sodium sulfate (pure)	5 Gm.
Salt (pure)	100 Gm.
Clear tap water containing no organic impurities	1,000 Gm.
Glycerin	50 Gm.

The solution is clear, practically colorless. It may be yellowish if the glycerin is yellowish. Icteric organs sometimes give it an icteric tint. After a couple of years it may become slightly yellowish, especially when the specimen is exposed to much light.

A few drops of a saturated camphor solution in 96 per cent alcohol is added. The white precipitate that forms is dissipated by superficial stirring with a glass rod. Then the glass cover is sealed on the container.

If the seal loosens and molds form, and even if the color of the specimen is spoiled on account of the molds, it is sufficient to leave the specimen for half an hour to a few hours in running water and in a jar into which a few crystals of potassium permanganate have been placed. The organ is then rinsed in running water, treated with salt water or not, replaced in fresh Hamdi solution and the jar resealed.

The advantages of Hamdi's solution are: It does not necessitate a preliminary treatment of specimens with alcohol as with most of the preserving solutions now in use. It preserves the specimens practically in their natural condition; it does not destroy the red blood corpuscles.

SUMMARY

An economical method of preserving pathologic and anatomic museum specimens is described.

From the Laboratory for Pathological Aanatomy, Gureba Hospital.

General Reviews

EXPERIMENTAL GASTRIC CARCINOMA

A CRITICAL REVIEW WITH COMMENTS ON THE CRITERIA OF INDUCED MALIGNANCY

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AND
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The production of experimental cancers is now generally considered to have started in 1914 with the finding of Yamagiwa and Ichikawa that repeated applications of coal tar to a rabbit's ear was followed in some cases by carcinomatous changes with metastasis. Since that time many different neoplasms have been produced experimentally by various methods. The studies of Kennaway, Cook, their co-workers and others in the isolation of a carcinogenic hydrocarbon from tar and later in the preparation and synthesis of other carcinogenic compounds opened up new methods of approach. In view of the rapid strides made in the past twenty-five years in the field of experimental cancer, it seems of interest to review what success has attended efforts to produce experimentally one of the most common malignant growths found in man—carcinoma of the stomach.[1]

In animals, carcinoma of the stomach is one of the rarest spontaneous cancers. Feldman [2] estimates that 8 to 10 per cent of old dogs have neoplasms, of which 40 to 50 per cent are malignant; yet all studies of the occurrence of spontaneous cancers in domestic, laboratory and wild animals uniformly show that carcinoma of the stomach is rare (Wells, Slye and Holmes [3]; Slye, Holmes and Wells [4]). There is no strain of animal in which gastric cancer develops regularly or indeed with sufficient frequency to warrant an attempt to develop a "cancer strain". Furthermore, the diagnosis for animals is almost invariably made post mortem.

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This review is preliminary to work aided by a grant from the Fenger Memorial Fund.

1. References to some work, particularly that pertaining to parasitic tumors, are chiefly to key articles. If the same paper has been published in several languages or journals, only one reference is usually made to it.

2. Feldman, W. H.: Neoplasms of Domesticated Animals, Philadelphia, W. B. Saunders Company, 1932, p. 68.

3. Wells, G. H.; Slye, M., and Holmes, H. F.: Am. J. Cancer **33**:223, 1938.

4. Slye, M.; Holmes, H. F., and Wells, H. G.: J. Cancer Research **2**:401, 1917.

In over 142,000 mice of the Slye stock, dying of natural causes, Wells, Slye and Holmes [3] reported 12 gastric cancers: 8 squamous cell carcinomas, 3 adenocarcinomas and 1 sarcoma. In addition there were 2 adenomas which may have been early adenocarcinomas. This represents all the gastric cancers in the Slye stock of mice in over twenty years of careful observation. This report, essentially confirmed by all other investigators, stresses the rarity of spontaneous gastric neoplasms in mice.

Stewart and Andervont [5a] found that in mice of both sexes of strain I there regularly develop spontaneous adenomatous, hypertrophic and hyperplastic overgrowths of the glandular rugae of the pyloric portion of the stomach with associated inflammatory changes and ulceration; metastases have not been observed. Histologic studies revealed some features somewhat suggestive of malignant growth. A few adenomatous tumors in the lungs were regarded as nonmetastatic, since in mice of this strain such pulmonary tumors develop spontaneously. Many etiologic factors were given serious consideration by these investigators, but no positive conclusion was reached. The condition, it is pointed out, bears some analogy to a rare gastric adenopapiliomatosis occurring in man and apparently having a familial tendency.

In marked contrast to the rarity of gastric cancer in animals is the well known frequency of carcinoma of the stomach in man. Livingston and Pack [6] estimated that 40,000 to 50,000 deaths are caused annually in the United States by gastric carcinoma. This is from 25 to 30 per cent of deaths due to malignant tumors, or about 3 per cent of all deaths. The mortality statistics of some European countries show that more than one half of all malignant neoplasms are carcinoma of the stomach (Konjetzny [7]).

In attempting to explain the marked difference between man and the lower animals in the development of spontaneous gastric cancer, attention naturally centered on the diet and on the fact that man is the only animal to heat his food and to consume it hot. In 1916 Lerche [8] analyzed the situation somewhat as follows: He reviewed statistics of the sites of carcinoma in the esophagus and stomach and the sites of cicatricial strictures resulting from swallowing corrosive fluids. The predisposition of certain sites to stricture formation following the ingestion of corrosive fluids was found to depend on the time of contact of the fluid with the various portions of the esophagus and stomach.

5. (*a*) Stewart, H. L., and Andervont, H. B.: Arch. Path. **26**:1009, 1938. (*b*) Stewart, H. L.: ibid. **29**:153, 1940.

6. Livingston, E. M., and Pack, G. T.: End Results in the Treatment of Gastric Carcinoma, New York, Paul B. Hoeber, Inc., 1939.

7. Konjetzny, G. E.: Der Magenkrebs, Stuttgart, Ferdinand Enke, 1938.

8. Lerche, W.: Surg., Gynec. & Obst. **23**:42, 1916.

Evidence was presented to show why cicatricial strictures do not occur at the sites of the physiologic narrowings but above them and why in cases in which a small amount of corrosive fluid was taken the esophagus escaped stricture formation entirely and only the antral area of the stomach was involved. The latter involvement was more frequent in nonfatal cases. It is significant that the favorite sites of carcinoma of the esophagus and stomach were found to correspond exactly to the favorite sites of cicatricial strictures in these organs. Since the explanation of the localization of corrosive action to certain areas depended on the mechanics involved when a fluid is ingested, it seemed reasonable to suspect some fluid used by man and not by animals as an etiologic factor in the production of carcinoma of the esophagus and stomach. Alcohol and other fluids were considered, but hot liquids remained as the most likely possibility.

Bonne [9c] had the unique opportunity to study two groups living in the same country with a marked difference in the incidence of gastric carcinoma: the Malays in Java, in whom gastric carcinoma is almost unknown, and the immigrated Chinese in Java, in whom it is not uncommon. In 3,885 autopsies on these Malays over a period of fifteen years, about three fourths of whom were males, only 1 gastric carcinoma was found, although the total cancer rate when recalculated for a population of standard age was found to be in accord with the usual figures for Western countries. Bonne and co-workers [9d] found no significant difference in the morphologic character of the stomach or in the gastric secretion in these two groups. They carefully studied the dietary and other habits but were unable to draw definite conclusions. Somewhat similar studies have been reported repeatedly, for example, those of Herbert and Bruske [10] and Lintott,[11] who, in attempting to account for the fact that the incidence of gastric carcinoma in Holland was twice that in England, concluded that an extrinsic factor was involved.

EXPERIMENTAL GASTRIC CARCINOMA

The animals almost universally used in reported investigations of experimental gastric carcinoma are mice and rats. Both the mouse and the rat have a stomach consisting of two chambers: (1) a forestomach comprising two thirds of the entire stomach and lined by squamous epithelium continuous with the esophagus and (2) a glandular stomach

9. (*a*) Bonne, C.: J. Trop. Med. **29**:288, 1926; (*b*) Ztschr. f. Krebsforsch. **25**:1, 1927; (*c*) Am. J. Cancer **30**:435, 1937. (*d*) Bonne, C.; Hartz, H.; Klerks, J. V.; Posthuma, J. H.; Radsma, W., and Tjokronegoro, S.: ibid. **33**:265, 1938. (*e*) Bonne, C., and Sandground, J. H.: ibid. **37**:173, 1939.

10. Herbert, W. E., and Bruske, J. S.: Guy's Hosp. Rep. **86**:301, 1936.

11. Lintott, G. A. M.: Guy's Hosp. Rep. **86**:293, 1936.

separated from the upper squamous-lined portion by a slight elevation, the limiting ridge.

Obviously two entirely different types of carcinoma might develop: (1) a squamous cell carcinoma of the nonsecreting forestomach, which would appear to be more closely related to a carcinoma of the esophagus or skin than to (2) an adenocarcinoma of the secreting glandular stomach. A sharp distinction between these two unrelated types should be made.

The means used in attempts to produce experimental gastric carcinoma generally fall into one of the following categories: (1) dietetic irritants, (2) parasites, (3) dietary deficiencies, and (4) agents known or suspected to be carcinogenic when fed, injected or implanted.

Dietetic Irritants.—The only attempt to produce gastric cancer experimentally by hot food was made by Lewis [12] a good many years ago. He poured hot mush daily into the gastrostomies of 35 dogs. Unfortunately, none of the dogs survived this procedure more than one year. No evidence of gastric carcinoma, gastric ulcer or gastritis was found. If this procedure could lead to changes eventually resulting in gastric carcinoma, it is now known, as pointed out by Wells, Slye and Holmes,[3] that it would probably require a longer time than these dogs survived. Experimental cancers of any kind are rare in dogs, but Passey's [13a] recent observation of a malignant melanoma occurring in a dog after six and one-half years of weekly tarring may give some indication of the induction period required.

Parasites.—The earliest attempts at experimental production of cancer to attract wide attention were those of Fibiger, who in 1913 published the first of a series of beautifully illustrated reports on the production of squamous cell carcinoma in the forestomachs of rats by means of a nematode, Gongylonema neoplasticum.[14] Much of this work has been criticized and remains unconfirmed (Passey, Leese and Knox [13b,c]; Cramer [15]).

Fibiger found squamous cell tumors in the forestomachs of wild rats infected with Gongylonema neoplasticum (Spiroptera neoplastica). Rats contracted the infection from cockroaches. Fibiger fed infected cockroaches to black and white laboratory rats and maintained them on a diet of white bread and water. After infection none of the rats lived more than two hundred and ninety-eight days. The changes in the stomach were limited to the squamous-lined forestomach. The glandular portion was never involved either primarily or by invasion. The stomach was often

12. Lewis, J. H., cited by Wells, Slye and Holmes.[3]

13. (*a*) Passey, R. D.: J. Path. & Bact. **47**:349, 1938. (*b*) Passey, R. D.; Leese, A., and Knox, J. C.: ibid. **40**:198, 1935; (*c*) **42**:425, 1936.

14. Fibiger, J.: Ztschr. f. Krebsforsch. (*a*) **13**:217, 1913; (*b*) **14**:295, 1914; (*c*) J. Cancer Research **4**:367, 1919.

15. Cramer, W.: Am. J. Cancer **31**:537, 1937.

enlarged, with nodular prominences on the external surface. The gastric mucosa had obvious papillary growths, and metastases were reported in the lungs of some rats. Microscopically, simple hyperplasia, hyperkeratosis and slight papillary downgrowths of the squamous epithelium were found in almost all rats. These changes were regarded as benign. In addition, over 50 per cent of the rats had changes interpreted as carcinomatous. In the first 111 rats reported on, Fibiger found, according to his criteria, 19 cases of squamous cell carcinoma, and later he observed similar cases in other rats. The changes in the forestomach were classified as malignant when the following conditions were present: (1) downgrowth of atypical and keratinized epithelial cells in abundance, arranged as spherical masses and horny globes; (2) infiltrative growths of these into the deeper layers, splitting up invasively the connective tissue of the mucosa and the muscle cells of the muscularis mucosae and also penetrating into the superficial and deeper layers of the submucosa. Neither infiltration of the muscularis or neighboring structures nor metastases were required for the designation of the tumors as malignant. As a matter of fact, the muscularis was not invaded even in those cases in which metastases were reported. Whether these represented actual metastases to the abdominal lymph nodes and the lungs is open to serious doubt. Lymph nodes similar to those believed by Fibiger to have metastatic deposits have been observed in the absence of malignant growth. Lesions in the lungs similar to those regarded as metastases have been seen repeatedly in rats without gastric lesions. They are usually bronchiectatic cavities or metaplastic epithelium.[13c]

As Cramer [15] recently pointed out in an excellent review of the subject, Fibiger's results have rarely been questioned, although many investigators seriously doubt his interpretation of the lesions as malignant. In the final analysis, much depends on whether metastases took place. Fibiger pointed out that the metastases to the lymph nodes were only microscopic, but the microscopic appearance of the so-called metastases is not conclusive.

Fibiger [14c] later reported a squamous cell carcinoma in the forestomach of a mouse 20 to 22 months old which died four hundred and twenty-eight days after Spiroptera infection. The tumor involved all layers of the stomach and invaded the liver, spleen and diaphragm. Numerous metastases were found in the abdomen. The metastases were successfully transplanted through four generations covering a period of one year, in the cases of 28 of the 55 mice that survived inoculation, and maintained the histologic features of the original gastric tumor. Fibiger regarded this as the same type of lesion he had observed in rats and emphasized the fact that this mouse lived more than four months longer, after Spiroptera infection, than any rat and that therefore the advanced characteristics of a malignant tumor

had time to develop. Passey, Leese and Knox,[13b] however, succeeded in keeping rats on a complete diet alive for four hundred and eighty days after infection with Gongylonema neoplasticum and observed only slight mucosal changes in the forestomach. This suggests that the Spiroptera infection in itself is not sufficient to cause the gastric lesions. There is little doubt that the gastric tumor in the mouse reported by Fibiger was a squamous cell carcinoma, for the muscularis and neighboring organs were invaded, and transplantation of the metastases was successful. Fibiger never reported a similar tumor in a rat, although a few similar but not as well established ones were found in mice.

Passey, Leese and Knox [13b] recently repeated Fibiger's experiments. As already mentioned, in a group of rats on a complete diet infected with Gongylonema neoplasticum the mucosal changes in the forestomach were insignificant. In a group of rats fed Fibiger's diet of white bread and water some lesions occurred in the forestomach, although they were not as severe as those seen in a similar group infected with Gongylonema neoplasticum. No gastric carcinoma, however, was found in any rat. Similar results were obtained in rats on a diet deficient in vitamin A with added parasitic infection. These investigators pointed out that Brumpt had observed only 1 gastric carcinoma in 781 wild rats found infected with Gongylonema neoplasticum and had failed to obtain a single carcinoma in 31 laboratory rats and 5 white mice experimentally infected with the parasite.

Bullock and Rohdenburg [16] believed that the lesions of the forestomach observed by Fibiger were benign and the result of nonspecific chronic irritation. They devised many ingenious experiments to test this hypothesis. They were unable to duplicate Fibiger's results exactly but produced a close resemblance. Three types of gastric irritation were used: (1) mechanical, (2) chemical and (3) a combination of the two.

A celluloid ball covered with pig's bristles was introduced into the rat's forestomach through a gastrotomy and suspended by a string. Within a few weeks irregular polypoid growths were observed, most marked at the site of incision. In control experiments, however, in which a simple purse string suture was taken in the stomach wall, without making an incision, the same changes were sometimes noted. In addition such polypoid growths were seen in some rats that were not operated on. When a cork ball with protruding pinpoints, covered with celluloid and keratin, was introduced into the stomach through a gastrotomy and allowed to remain free in the gastric cavity, lesions were usually found in the glandular chamber of the stomach and were more severe when the gastrotomy had been in the glandular portion. The

16. Bullock, F. D., and Rohdenburg, G. L.: J. Cancer Research **3**:227, 1918

changes consisted of an increase in the mucous cells and a complete absence or scarcity of the parietal cells with downgrowths of glandular epithelium forming cystadenomas and accompanied by varing degrees of inflammation of the gastric wall. It is interesting that in some cases osteoid tissue was found in the connective tissue of the stratum proprium. When a rubber sponge impregnated with scarlet red powder or pine tar was introduced into the stomach, hypertrophy and proliferation of the squamous epithelium, especially around the gastrotomy, took place and sometimes penetrated the muscularis mucosae. Similar changes were found after scarlet red or pine tar in oil or ether had been injected directly into the stomach wall. Although the factor of a food deficiency may have played some role in these results, Bullock and Rohdenburg clearly showed that lesions of the forestomach resembling those observed by Fibiger could be produced in the absence of parasites. Furthermore, the well illustrated benign cystadenomatous lesions in the glandular stomach form an instructive basis of comparison with similar lesions which have at times been reported as malignant.

Yokogawa [17] reported the experimental production of squamous cell carcinoma in the forestomach of the rat by means of another nematode, Gongylonema orientale. Carcinoma was reported to have developed in 3 of 61 white rats after at least two hundred days of infection. The characteristics of these tumors were in all essential respects similar to those observed by Fibiger. The tumors were papillomatous growths which at their base extended down through the muscularis mucosae into the submucosa but did not involve the muscularis. Invasion of lymphatic spaces was present. The glandular portion of the stomach showed some atrophic change but was otherwise not involved. Metastases to the liver and lung were reported in one of the rats. Yokogawa described these metastases grossly as hard grayish spots containing a cheesy material. Microscopically, the cavities were lined in part by laminated flat cells. Yokogawa recognized that such lesions were sometimes found in rats without cancer but thought the evidence in this particular case pointed to metastases. A photomicrograph of one such lesion leaves some doubt whether this was actually a metastasis. Yokogawa's observations are essentially similar to those of Fibiger, and their interpretation is open to the same objections.

Bonne [9a] reported a squamous cell tumor in the forestomach of a wild rat caught in Italy and found infected with a nematode, Hepaticola gastrica. The tumor was a papilloma the size of a cherry. Microscopically, the muscularis mucosae was penetrated by an epithelial downgrowth, but the muscularis was uninvolved. The glandular stomach was normal. No metastasis was found. Evidence that this was

17. Yokogawa, S.: Gann **18**:48, 1925.

a malignant growth rests chiefly on the fact that the tumor invaded some blood vessels. Bonne pointed out the close resemblance to Fibiger's tumors and the probability that the nematode infection of the stomach was casual rather than coincidental. Whether this represented a true cancer of the forestomach can be questioned.

Vogel [18] later experimentally inoculated rats with Hepaticola gastrica. Although he failed to obtain infection of old rats, he succeeded in producing infections in 4 young ones, in 2 of which squamous cell carcinoma of the forestomach was reported to have developed after three months. Vogel noted that there was considerable similarity to the lesions observed by Fibiger with the exception of a greater tendency toward epithelial downgrowth into the submucosa and a lesser amount of papillomatosis into the lumen. The muscularis was not involved, and the glandular stomach was normal. Metastases are not mentioned. Vogel stressed the fact that no parasites were found in the tumors when the animals died and regarded this as evidence that a true malignant growth was present in that after the malignant change had taken place it persisted without the presence of the casual agent. Although this is one explanation it is not by any means the only one.

Bonne and Sandground [9e] recently noted the association of a "gastric adenoma bordering on malignancy" and a nematode, Nochtia nochti, in Javanese monkeys. The adenoma was never observed in the absence of the parasite, and whenever the parasite was found, an adenoma was present. These authors succeeded in inoculating monkeys with Nochtia nochti and producing such tumors experimentally. No metastases were observed, but definite invasion of blood vessels was noted. Whether these adenomas can develop into adenocarcinomas remains an open question.

Comment: A review of gastric parasitic infections does not disclose conclusive evidence that infection in itself leads to gastric tumors in spontaneously or experimentally infected rats. The tumors observed in such animals may be due primarily to other factors not satisfactorily eliminated in the experiments, such as dietary deficiencies. When Passey, Lesse and Knox [13b] inoculated rats maintained on a complete diet with Spiroptera neoplastica, insignificant gastric mucosal changes resulted. It is therefore not surprising that some investigators found more severe gastric lesions in young and growing rats than in old ones. In the interpretation of reports, due allowance should be made for the variation of different species and strains of animals in their reaction to the same dietary deficiency or parasite. The role of nematode infections in the genesis of forestomach lesions in rats seems to be that of aggravating mucosal changes initiated by other causes, although parasites,

18. Vogel, H.: Ztschr. f. Krebsforsch. **29**:351, 1929.

as is well known, can cause tissue proliferation and malignant growth in other organs. Hoepple,[19] in a comprehensive review of parasites and tumor growth, discussed the various mechanisms by which this may occur. Bonne and Sandground's [9e] observations indicate, however, that in monkeys inoculated with Nochtia nochti gastric adenomas occur only in the presence of parasitic infection.

Regardless of the exact cause of the gastric squamous cell tumors attributed to parasites, it is of interest to consider the evidence of the malignant nature of these lesions. Fibiger's [14c] clearly stated criteria of malignancy, previously mentioned, will be more fully considered later. It is, however, generally accepted at present that his criteria are inadequate. Whether such changes can be regarded as "precancerous" will remain an open question until it is possible to observe more frequent transition to undoubted malignancy. Fibiger reported squamous cell tumors in a few mice, one of which was supported by practically conclusive evidence of malignancy; however, in view of the large number of animals used in Fibiger's work the possibility of spontaneous malignancy was not entirely eliminated. The causal relation between gastric squamous cell carcinoma, parasites and other factors that may have been unknowingly involved needs to be demonstrated more regularly to be convincing.

Dietary Deficiencies.—Dietary deficiencies may seem unrelated to experimental gastric carcinoma, but the severe changes in the forestomach apparently caused by such deficiencies, particularly in rats, have been reported at times as malignant. Furthermore, because these severe changes closely simulate those sometimes interpreted as carcinoma and attributed to other causes, a food deficiency should be considered as a possible factor in some of the reports of experimental gastric carcinoma.

Pappenheimer and Larimore,[20a] in 1923, were the first to call attention to the possible relation of forestomach lesions in rats to a dietary deficiency. Their preliminary evidence suggested that vitamin A deficiency might be the cause. Further study,[20b] however, revealed that the lesions were present when sufficient ultraviolet radiation was given to prevent rickets. The lesions were uncommon in rats on a complete diet, and when present, were mild. When rats were placed on a diet known to bring about the forestomach lesions, neither the addition of cod liver oil or of Osborne and Wakeman's yeast extract or changes in the mineral content of the diet prevented them. Pappenheimer and Larimore concluded that a food deficiency was a factor in the appearance

19. Hoepple, R.: Chinese M. J. **47**:1075, 1933.

20. Pappenheimer, A. M., and Larimore, L. D.: (*a*) Proc. Soc. Exper. Biol. & Med. **21**:141, 1923-1924; (*b*) J. Exper. Med. **40**:719, 1924.

of the gastric lesions but were unable to determine what element of diet was lacking. They observed that rats on a deficient diet often had hair in their stomachs and suggested that this hair might aggravate the lesions. Of 6 rats fed a complete diet mixed with ground-up hair, 2 were found to have benign changes of the forestomach and ulcers, with hair embedded in the stomach in such positions as to indicate that it was a factor in the production of the ulcers. The lesions observed by Pappenheimer and Larimore were limited to the forestomach, usually to an area near the elevated ridge separating the forestomach from the glandular stomach, and consisted essentially of papillomatosis. The changes were considered benign.

Wolbach and Howe [21] in 1925 reported change caused by vitamin A deficiency in rats. They took precautions to prevent any deficiency other than vitamin A by forced feeding whenever necessary, particularly after anorexia and impairment of the sense of smell developed. No significant gastric lesion was observed in spite of extensive changes in other covering epitheliums. Wolbach and Howe found large bronchiectatic cavities in many of the rats, which were often considered the immediate cause of death. Although tissue changes in the stomach were not found, the alteration in the covering epithelium caused by vitamin A deficiency is worth noting: "Growth activity of the epithelium is not diminished; on the contrary, there is convincing evidence that it is greatly augmented. In a few of our animals, the behavior of the replacing epithelium in respect to numbers of mitotic figures and response on the part of connective tissue and blood vessels suggests the acquisition of neoplastic properties." Because of the thoroughness of this work, the observations in relation to the stomach and lungs seem particularly significant. It has been suggested that the so-called metastases to the lungs in rats with gastric tumors were sometimes bronchiectatic cavities. That such cavities do appear in rats without any pathologic changes in the stomach was clearly shown by Wolbach and Howe and has been repeatedly confirmed by others.

Fujimaki [22a, b] in 1926 observed severe papillomatosis of the forestomach in young rats on a vitamin A–deficient diet. The lesions were believed to be malignant. One of 5 rats with the most severe gastric papillomatosis was reported to have metastatic nodules in the lung. One of these metastases is illustrated.[22b] The gastric lesions consisted essentially of hyperkeratosis and downgrowth of atypical epithelium with penetration of the muscularis mucosae. The muscularis was not invaded. The glandular stomach was normal. An accompanying editorial note

21. Wolbach, S. B., and Howe, P. R.: J. Exper. Med. **42**:753, 1925.

22. (*a*) Fujimaki, Y.: J. Cancer Research **10**:469, 1926; (*b*) Gann **21**:8, 1927. (*c*) Fujimaki, Y.; Arimoto, K.; Kimura, T.; Ohba, K., and Matsuda, G.: Tr. Jap. Path. Soc. **21**:708, 1931.

states that microscopic examination of Fujimaki's slides, from which the photomicrographs were prepared, left some doubt as to the malignant nature of the changes.[22a] In a more recent publication Fujimaki and co-workers [22c] offered evidence to show that various fatty acids and lipoids in the diet are factors in the appearance of atypical epithelial proliferation of the forestomach in rats and that vitamin A (butter) does not prevent it.

Cramer's [15] experiments with vitamin A–deficient rats are worthy of note for two reasons. First, the severe papillomatosis of the forestomach was considered benign, and, second, two experiments done ten years apart, with apparently identical methods except that different strains of rats were used, showed a startling difference in results. Cramer stated that if the earlier sections of the stomach had not been available for reexamination he himself could hardly have believed that the benign papillomatosis, as marked as that observed by Fibiger, could have been so severe in the first experiment and so mild in the second. He has no explanation to offer except that different strains of rats react in varying degrees to vitamin A deficiency or that the papillomatosis of the forestomach may be due to a virus the action of which is enhanced in rats on a deficient diet.

Hoelzel and Da Costa [23] offered a hypothesis of the genesis of the forestomach lesions in rats observed by them without postulating any specific food deficiency. They found that rats on a low protein diet when fed alternately for two days and starved for two days had ulcers of the forestomach after two weeks. These authors stressed the presence of the ulcers, although some papillomatosis apparently also was present. The simplest method of producing such lesions was found to be starvation. Inanition alone, however, did not cause the ulcers to persist, as shown by the fact that they healed readily on a bran diet which had no caloric value. Ulcers also developed in rats on a bread diet. The incidence of the ulcers decreased when 15 to 30 per cent calcium carbonate was added to the bread. Ingestion of hair seemed to cause ulceration only when this interfered with a normal intake of food. Their observations led them to conclude that the acid-combining properties of the food largely determined the amount of ulceration and that the acid gastric juice probably acted as an irritant in the manner shown by Bullock and Rohdenburg [16] by artificial irritation.

Rats fed a high fat, low protein diet by Hoelzel and Da Costa [23b] were found to have a particularly prominent overgrowth of the mucosa of the forestomach. This observation is similar to that of Fujimaki and co-workers,[22c] who found that an increase in papillomatosis resulted in

23. Hoelzel, F., and Da Costa, E.: Proc. Soc. Exper. Biol. & Med. (*a*) **29**: 382, 1931; (*b*) **29**:385, 1932.

rats fed various fatty acids and lipoids. Because these lesions resembled those previously reported by some investigators as malignant, Hoelzel and Da Costa made a study of the morphologic changes and found that grossly they consisted of nodular prominences on the outer surface of the stomach with ulceration and overgrowth of the mucosa. Microscopically, the largest nodules were found to be epithelial cysts, generally located at the limiting ridge. Other nodules consisted of cornified globular masses. In most cases continuity with the normal epithelial layer could be demonstrated. Although there was a downward growth of the epithelium, the muscularis was never invaded. Observations on 7 rats which were fed alternately in a manner that produced ulcers and nodules in control rats and which then were given a complete diet indicated that these rather severe changes were reversible. The ulcers disappeared faster than the nodules or epithelial cysts, but even these seemed to disappear eventually. Hoelzel and Da Costa concluded that the lesions were not malignant. It is unknown whether they would have become malignant in a more prolonged experiment.

Harde [24] reported a squamous cell carcinoma in the forestomach of a mouse fed a deficient diet for seven months. Because of a paratyphoid infection at the end of this time, a complete diet was given and continued until the mouse died seven months later. A squamous cell tumor, 1 by 2 cm., was found in the forestomach, which infiltrated all layers of the gastric wall and invaded the liver. In addition there was adenomatosis of the glandular stomach, which infiltrated the submucosa. Whether metastases were present is not known. There is no illustration. Harde made no attempt to correlate the experimental procedure with the appearance of the carcinoma since this was the only cancer in over 600 mice.

Findlay [25] in 1928 reported lesions in rats due to vitamin B_1 and vitamin B_2 deficiency. He found papillomatosis, consisting of thick layers of squamous cells with exfoliation of the keratin layer, in the forestomach of every rat fed a diet deficient in vitamin B_2. The epithelium formed papillary projections into the lumen, and blunter processes extended downward as far as the muscularis mucosae. There were numerous mitotic figures. Ulceration of the superficial layers of the mucosa was present. No evidence of malignancy was found. Such gastric changes were never present in rats on vitamin B_1 deficiency only. Findlay agreed with Pappenheimer and Larimore that the ingestion of hair might afford a plausible explanation of the lesions, since vitamin B_2–deficient rats shed large amounts, which inevitably contaminated the diet. Among other lesions found in vitamin B_2–deficient rats, Findlay

24. Harde, E.: Compt. rend. Soc. de biol. **110**:245, 1932.
25. Findlay, G. M.: J. Path. & Bact. **31**:353, 1928.

reported profound changes in the squamous epithelium of the skin, consisting of active mitosis in the cells of the stratum granulosum, while the whole process of keratinization was excessively active. All the changes are well illustrated.

Sharpless [26a] observed lesions developing in the forestomachs of rats within three months after they were placed on a low casein diet. These lesions were described essentially as thickening of the epithelial ridges with papillomatosis, hyperkeratosis and ulceration of the mucosa, most marked along the limiting ridge. No metastases were observed.

In a later report Sharpless [26b] described hyperplastic changes of the forestomach in all of the 125 rats fed a low casein diet and no such lesion in 200 control rats on a complete diet. This stock of rats has shown no spontaneous malignant tumor in over four years. Control rats fed the same diet with the addition of 12 per cent vitamin-free casein or 0.2 per cent cystine showed no lesions. When given a low casein diet with the addition of 10 per cent gelatin, to approximate the physical properties of a diet without a protein deficiency, the lesions appeared as though a low casein diet only had been fed. The papillomatosis in some instances almost occluded the lumen. In cases of long standing, spurs of atypical, keratinizing epithelium sometimes penetrated the muscularis mucosae, extending into the submucosa, and proliferated, forming cysts. Active invasion of the muscularis was reported in some rats. As many as eighteen mitotic figures were observed in one high power field. No metastases were found. One of several photomicrographs has a legend suggesting a malignant process. Sharpless regarded some of these lesions as malignant; however, the evidence has been considered inadequate.[26c]

Howes and Vivier [27] reviewed the relation of diet to the occurrence of gastric lesions in rats and the interpretation of these changes. In their own work they found that whole yeast prevented the forestomach lesions when added to the diet used by Pappenheimer and Larimore to produce them, whereas the addition of Osborne and Wakeman's yeast extract, vitamin-free casein, cod liver oil or other forms of vitamin A was of no protective value. The gastric changes observed when whole yeast was not added to the diet were essentially hyperplasia, hyperkeratinization and ulceration of the squamous epithelium in the forestomach, with hypertrophy of the limiting ridge. Similar lesions without keratinization were found in the glandular stomach. Mitotic figures were numerous. The muscularis mucosae was thinned but intact. Hair was found in the stomachs of only 2 rats and therefore was not regarded

26. Sharpless, G. R.: (*a*) Proc. Soc. Exper. Biol. & Med. **34**:684, 1936; (*b*) Ann. Surg. **106**:562, 1937; (*c*) abstracted, Am. J. Cancer **37**:457, 1939.

27. Howes, E. L., and Vivier, P. J.: Am. J. Path. **12**:689, 1936.

as the primary cause of the lesions. Pappenheimer and Larimore's failure to prevent the lesions with Osborne and Wakeman's yeast extract was regarded as due to the lack of something which is present in whole yeast. Their explanation of the apparently conflicting views of the role of vitamin A deficiency was that Wolbach and Howe had previously observed no gastric lesions in rats on a diet deficient only in vitamin A. Since anorexia is a prominent symptom in vitamin A deficiency, secondary deficiencies are likely to develop. Howes and Vivier believed these secondary deficiencies to be a factor in the appearance of the lesions.

Comment: A review of the gastric lesions present in animals on various deficient diets, particularly those in the forestomachs of rats, does not disclose sufficient evidence to warrant the conclusion that they are definitely malignant. There is a striking similarity between the forestomach changes in rats on deficient diets and those observed in rats with nematode infections. This resemblance is so close that it has been suggested a dietary deficiency is the primary cause of the parasitic tumors. Whether a specific deficiency is responsible for the lesions is still a controversial matter. Cramer [15] suggested a possible mechanism by which dietary deficiencies may lead to papillomatosis of the forestomach. He reviewed the conflicting evidence of its cause appearing in the literature and the highly inconsistent results in his own experiments, previously mentioned. Although different strains of animals vary in their reaction to the same deficiency, this may not adequately account for all observations. The fact that some cutaneous papillomas can be transmitted by means of a virus led Cramer to speculate that the primary cause of papillomatosis of the forestomach may possibly be a virus whose action is enhanced in rats fed a deficient diet. This hypothesis in one form or another has been previously suggested by others, but Cramer's experiments are an example of its possibility and might explain, among other things, the suspected occasional endemic occurrence of forestomach papillomatosis in rats.

Lesions of the glandular stomach have been observed in rats on deficient diets [27] but much less commonly than those of the forestomach, and they have not been regarded as malignant.

A question of prime importance in relation to gastric lesions resulting from dietary deficiencies of one sort or another is that of the reversibility of the changes. This possibility has not been satisfactorily eliminated in the more severe lesions sometimes regarded as malignant. It seems essential to determine whether the gastric changes will persist and progress invasively after the resumption of a complete diet. The evidence presented by Hoelzel and Da Costa [23b] indicates that the lesions they observed, possibly not as severe but apparently of the same type as those sometimes simulating malignant growth, are reversible.

Carcinogenic Agents.—1. Tar. With the advent of tar as a carcinogenic agent, attention was directed primarily to experimental tumors in organs or tissues other than the stomach, although some attempts to produce gastric carcinoma with tar have been reported. Ishibashi and Ohtani [28] found definite adenomas, not regarded as malignant, fifty days after the injection of tar into the submucosa of the stomach in rabbits.

Buschke and Langer [29] noted forestomach lesions in 50 of 54 rats after rectal injections of tar once to twice a week for four to six months. The animals were 3 to 5 months old at the beginning of the experiment. The first rectal injection led to rather severe general reactions, although subsequent injections were well tolerated for the next two to three months, and then there was a progressive decline in general health. The animals were allowed to die spontaneously. The lesions were limited to the forestomach and were usually near the ridge separating it from the glandular chamber. The rectum, glandular stomach and intestine showed no significant changes. An excellent description and photographs of the lesions bear out the close resemblance, as pointed out by the authors, to those noted by Fibiger. The changes consisted essentially of ulceration, hyperkeratosis and papillomatosis of the mucosa with a downgrowth of the epithelium, sometimes extending through the muscularis mucosae into the submucosa and part of the muscularis but never through the serosa. Mitotic figures were common. The careful pathologic analysis of the lesions is noteworthy. Although some regional lymph nodes were enlarged, no metastatic tissue was found microscopically nor were metastases found elsewhere. Nests of epithelium sometimes appeared to be isolated, but a connection with the surface epithelium was found. This was not always obvious, and the authors pointed out that the connection might easily have been overlooked in some sections. In addition, the epithelial downgrowths did not seem to infiltrate the deeper structures but rather to push through them, even to the extent of giving the external surface of the stomach a nodular appearance. In view of these observations the lesions were not considered malignant, although the possibility that in time a malignant change might have occurred was discussed. The relation of the rectal injections of tar to the lesions of the forestomach is of considerable interest. Buschke and Langer considered but dismissed the possibility that some tar was swallowed. No gastric parasites were found. They felt the changes were due to the general effect of absorbed tar. In retrospect it may be questioned whether the tar had any specific effect. The animals were allowed to die spontaneously and were reported to have been in a poor nutritional state for some time before death. Pre-

28. Ishibashi, M., and Ohtani, S.: Gann **15**:2, 1921.
29. Buschke, A., and Langer, E.: Ztschr. f. Krebsforsch. **21**:1, 1923.

sumably there was anorexia with consequent inadequate intake of food and dietary deficiencies such as have been found to lead to similar alterations in the stomach.

Bonne,[9b] in an excellent paper dealing in part with gastric lesions in tarred animals, found benign papillomas in the forestomachs of a few mice. Bonne thought these were probably caused by swallowed tar. When tar was applied to the mouths of 50 mice, the incidence of papillomas definitely increased, and 1 mouse was reported to have had a squamous cell carcinoma of the forestomach. Tar had been applied orally twice a week for thirty-three weeks and then discontinued. At the time of death, one year after tarring was started, this mouse had a carcinoma of the lower lip and buccal mucosa in addition to the gastric tumor, which penetrated the muscularis mucosae and infiltrated almost to the serosa. No metastasis was found. No further description or illustration of this tumor is given. No malignant gastric lesion was found in 20 rats repeatedly tarred by mouth. A papilloma in the forestomach of 1 rat and some benign changes in the glandular stomach of another were regarded as spontaneous, since similar lesions had been observed in untarred rats. A third rat tarred by mouth twice a week for twenty weeks was found to have bronchopneumonia and two grossly visible white nodules in the lung. Microscopic examination disclosed a large keratinized mass in the periphery of the lung. Squamous epithelium was recognizable at the borders of this mass, which was nowhere seen to be related to a bronchus. The remainder of the lung could not be examined for further squamous cell metaplasia. This finding is interesting, since no squamous cell tumor was found elsewhere to suggest a metastatic lesion in the lung. A photomicrograph beautifully illustrates the keratinized pulmonary mass.

Voronoff and Alexandrescu [30] fed 10 white rats a mixture of tar, hydrous wool fat, aniline oil and toluylenediamine three to four times a week. A peritoneal sarcoma and an adenocarcinoma of the prepyloric portion of the stomach were reported in a rat dying six months later. The latter tumor is described as infiltrating all layers of the stomach, with metastases to the liver and retropyloric lymph nodes. Drawings of the microscopic appearance of this lesion show the superficial gastric mucosa, not including the muscularis mucosae nor the deeper layers of the stomach. Some mucosal ulceration and various alterations of the glandular structure are evident, but the drawings are not clear enough to permit an objective interpretation. There is no illustration of a metastasis. This work apparently has not been repeated by the same or other investigators.

30. Voronoff, S., and Alexandrescu, G.: Néoplasmes **8**:129, 1929.

Tani [31] noted marked papillomatosis of the forestomach in a few rats given tar orally. The changes were severe enough to suggest a malignant process. Injections of tar into the gastric wall were found to be less effective. Microscopic studies were not found in the reports available.

Twort and Twort [32a] in a detailed report of lesions found in 60,000 tarred mice, state that while they were not primarily interested in the alimentary tracts of their animals and hence may have overlooked some gastric lesions, they noted occasional papillomas of the forestomach, more frequently in those mice painted with tar for many weeks than in those painted for only a short time. One mouse had a pronounced benign adenomatous or hyperplastic condition of the glandular portion of the stomach. It was, of course, impossible to say whether 1 glandular lesion in 60,000 mice was casually related to the tarring or to the swallowed tar. They found no gastric carcinoma in any of the 60,000 mice.

Mercier and Gosselin [33] injected coal tar in olive oil intraperitoneally into a mouse, which died four months later. A tumor the size of a large pea was found in the forestomach near the limiting ridge. It is briefly described as infiltrating the musculature. The tumor was considered malignant and was regarded as the result of irritation by swallowed hair contaminated with tar rather than of the intraperitoneal injection of coal tar in oil. It is not clear whether metastases were present. There is no illustration.

Reinhard and Candee [33a] fed mice 10 mg. of coal tar in butter once a week for seven months and observed no gastric tumors.

2. Carcinogenic Chemicals. With the isolation and synthesis of a carcinogenic constituent of tar and the preparation of many other carcinogenic agents, it became possible to feed animals single chemical compounds of known carcinogenic potency for some tissues and organs. In the meantime the carcinogenic properties of some other substances were recognized or suspected. Some of these have been used in attempts to induce gastric carcinoma in animals, usually by feeding, although other methods have been employed as well.

(*a*) Diaminoazobenzene: Otsuka [34] in 1935 noted papillomatosis of the forestomach in mice fed diaminoazobenzene in olive oil daily. A closely related compound, dimethylaminoazobenzene, has been used in

31. Tani, I.: Tr. Jap. Path. Soc. **21**:715, 1931.

32. (*a*) Twort, J. M., and Twort, C. C.: J. Path. & Bact. **35**:219, 1932. (*b*) Twort, C. C., and Bottomley, A. C.: Lancet **2**:776, 1932.

33. Mercier, L., and Gosselin, L.: Compt. rend. Soc. de biol. **113**:669, 1933.

33a. Reinhard, M. C., and Candee, C. F.: Am. J. Cancer **26**:552, 1936.

34. Otsuka, I.: Gann **29**:209, 1935.

the experimental production of carcinoma of the liver in rats but causes no malignant lesion in the stomach (Kinosita [35]). Diaminoazobenzene, on the other hand, has little effect on the liver. Otsuka observed papillomatosis as early as fifty-nine days after diaminoazobenzene was fed and found that it was present in all mice surviving three hundred and seven days or more. There was no invasion of the muscularis and no metastasis. The changes were considered benign. The glandular stomach was not involved.

(*b*) 1,2,5,6-Dibenzanthracene: Reports of gastric lesions following administration of 1,2,5,6-dibenzanthracene are uncommon.

Perry and Leonard [36] reported that carcinoma of the stomach developed in some mice painted twice a week with 0.3 per cent 1,2,5,6-dibenzanthracene in benzene. In mice painted with theelin in addition to dibenzanthracene more tumors developed, but it is not stated whether this included gastric tumors. It is not clear how long the mice with gastric tumors had been painted. There is no further description. No mention is made of the type of neoplasm—squamous or glandular. There is no illustration of a gastric tumor.

Reinhard and Candee [38a] fed mice 0.02 per cent dibenzanthracene in butter for seven months without obtaining gastric or other tumors.

Ilfield,[37] using Shear's method of incorporating carcinogenic hydrocarbons in cholesterol pellets, implanted 5 per cent dibenzanthracene pellets in the wall of the stomach of a dog. No tumor was found after one year. Similar pellets placed under the gastric serosa of ferrets did not cause a tumor at the time of report, four months later.

Cook and co-workers [38] reported that no carcinoma of the alimentary tract resulted from feeding dibenzanthracene in lard for an unspecified length of time to mice and rats.

Branch [39] painted the skin of mice twice a week with 0.5 per cent dibenzanthracene in benzene and noted considerable licking of the painted areas with the resultant ingestion of dibenzanthracene, but no tumor of the gastrointestinal tract was found in any mouse.

Van Prohaska, Brunschwig and Wilson [40] failed to obtain gastric lesions in white mice by feeding dibenzanthracene in lard three times a week for six months.

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35. Kinosita, R.: Tr. Jap. Path. Soc. **27**:665, 1937.

36. Perry, I., and Leonard, G. L.: Am. J. Cancer **29**:680, 1937.

37. Ilfield, F. W.: Am. J. Cancer **26**:743, 1936.

38. Cook, J. W.; Haslewood, G. A. D.; Hewitt, C. L.; Hieger, I.; Kennaway, E. L., and Mayneord, W. V.: Am. J. Cancer **29**:219, 1937.

39. Branch, C. F.: Am. J. Cancer **26**:110, 1936.

40. Van Prohaska, J.; Brunschwig, A., and Wilson, H.: Arch. Surg. **38**:328, 1939.

Fieser,[41] in a comprehensive review of the relative carcinogenic properties of various polynuclear aromatic hydrocarbons, found that 1,2,5,6-dibenzanthracene usually requires a longer time to induce skin and subcutaneous tumors in mice and rats than some of the others. The possibility that gastric lesions might result in animals fed dibenzanthracene for longer periods is therefore not entirely excluded.

(*c*) Cholesterol Compounds: Roffo[42a] reported the experimental production of adenocarcinoma of the stomach in rats. All rats were 3 months old at the beginning of the experiment and were maintained on a diet of bread and milk for sixteen to twenty-six months thereafter. A control group of rats fed this diet or bread, milk and unirradiated cholesterol showed no gastric lesions similar to those observed when various additions to the diet were made. The daily additions consisted of 100 mg. of forty-eight hour ultraviolet-irradiated cholesterol, heated cholesterol, sun-treated or ultraviolet-irradiated egg yolk. One group was fed ultraviolet-irradiated bread and milk. In all, 600 rats were used in these experiments. A typical illustration is given in each group, but it is not clear how many rats showed the gastric changes described. Roffo found the most common lesion to be ulceration in various forms and degrees in the glandular stomach and forestomach. The lesions in the glandular stomach are well illustrated. Some were regarded as ulcers in which carcinomatous degeneration had taken place.

In a more recent publication Roffo[42b, c] reported the results of feeding rats various animal fats (beef, pork or lamb) or olive oil heated to 350 C. for a half hour. This procedure is reported to have changed the cholesterol to oxycholesterin, which was found to have a fluorescence spectrum similar to some of the carcinogenic hydrocarbons. In other respects the experimental method was identical to that of the previous experiment. Malignant lesions of the stomach, liver and lung are described. Lesions of the glandular stomach far outnumbered all others, and the discussion will be limited to these. The gross findings in 200 rats are tabulated and show that over one third had some gastric lesion, most commonly multiple ulcers of the glandular stomach. In some rats gastric tumors (or polyposis) were present. Histologically, in addition to the ulcers, hyperplasia of the gastric mucosa, epithelial cysts (cystadenomas) and atypical glandular epithelium were described, and in some instances destruction of parts of the muscularis mucosae and of the muscularis was present. These changes are well shown in numerous photographs of gross specimens and in photomicrographs. Some of the more advanced lesions were regarded as adenocarcinomas.

41. Fieser, L. F.: Am. J. Cancer **34**:37, 1938.

42. Roffo, A. H.: (*a*) Ztschr. f. Krebsforsch. **47**:473, 1938; (*b*) Bull. Assoc. franç. p. l'étude du cancer **28**:556, 1939; (*c*) Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **15**:407, 1938.

Roffo used a total of 1,600 rats in cholesterol feeding experiments of one type or another. The number of lesions regarded as carcinomatous degeneration of ulcers or adenocarcinomas is not entirely clear, but certainly there were quite a few, and control experiments eliminated the possibility that these were coincidental. On the basis of the changes shown in the photomicrographs, the question may be seriously raised as to whether these lesions were really malignant. Aside from the profound changes in the glandular epithelium, some of the strongest evidence in favor of malignancy is found in the fact that in some cases the muscularis was destroyed in places by a downgrowth of atypical glandular tissue, cystadenomatous in form. The question arises whether a process limited in all cases by the gastric serosa can be considered malignant. In the absence of any mention of a metastasis from a gastric lesion or of an instance of invasion of a neighboring structure or organ, the evidence for malignancy is not entirely convincing. In this connection it is interesting to compare the photomicrographs of lesions regarded as adenocarcinomas by Roffo with the less severe but similar changes observed by Bullock and Rohdenburg [16] in rats after introducing into the stomachs cork balls with protruding pinpoints.

Waterman [43] fed tarred mice cholesterol oleate twice a week. The mortality was high. Forty mice survived sixty days or more. Twelve of these had benign forestomach papillomas, and 3 had what was interpreted as squamous cell carcinoma of the forestomach. The latter were found in mice that succumbed ninety-three, two hundred and forty-eight and two hundred and forty-eight days, respectively, after the feeding of cholesterol oleate. No further description is given. Metastases are not mentioned; presumably there were none. A low power photomicrograph illustrating a carcinoma shows a papilloma near the limiting ridge. No invasion of the muscularis can be seen. On the basis of this illustration it is difficult to differentiate the lesion from previously described benign papillomas. Waterman in another experiment fed 6 untarred mice cholesterol oleate three times a week. After three hundred and eighteen days he found a malignant papilloma in the forestomach and a fibrosarcoma in the neck of the stomach in 1 mouse. Another had a squamous cell carcinoma of the forestomach after four hundred and forty-one days. A third mouse had polypoid growths in the glandular stomach showing many mitoses after three hundred and sixty days. No metastases are mentioned, and no description of the lesions are given, although photographs of the gross and microscopic appearance accompany the report. These photographs are in no way convincing. A fourth mouse after four hundred and forty-one days was found to have

43. Waterman, N.: Acta cancrol. **2**:375, 1936.

a lesion regarded by Waterman as an early adenocarcinoma of the stomach without a metastasis. This is a most interesting interpretation. A description and illustrations of the lesion are given. It appears to be along the limiting ridge, depressed in relation to the surrounding mucosa, and might easily be overlooked on gross examination. The muscularis mucosae is not distinct. There is no invasion of the muscularis. A higher power photomicrograph taken from an unspecified part of the mucosa shows an acinar arrangement of apparently single layers of cells. The description and illustrations indicate there was some alteration in a small part of the glandular mucosa. That this represented a malignant change is, however, a matter of opinion not well supported by the evidence.

(*d*) 3,4-Benzpyrene: Oberling, Sannie, Guérin and Guérin [44] fed 20 mice 3,4-benzpyrene in lard once a week. Sixteen mice succumbed at the end of six months. One of these showed benign hyperplasia of the forestomach mucosa. None of the others had any appreciable gastric changes. Of 20 rats fed benzpyrene in lard, 10 died within seven months. None of these had any significant gastric lesion. The other 10 rats appeared in good health at the time of the report.

Ilfield [37] obtained negative results (at four months) by implanting 5 per cent benzpyrene in cholesterol pellets under the gastric serosa of ferrets.

Waterman [43] fed 6 mice 0.4 per cent 3,4-benzpyrene in lard daily for periods ranging from one hundred and twelve to three hundred and thirty-six days. When benzpyrene was given in watery colloidal solution, no gastric lesions were observed. With lard as the solvent, however, 5 mice had forestomach tumors that were regarded as squamous cell carcinoma. Metastases to the portal glands, peritoneum, liver, spleen or lungs were reported in 4 of these animals, but the several photographs of the lesions, including one of a reported metastatic nodule in the liver, are not convincing, and no microscopic description of the primary or metastatic lesions is given.

Rusch, Baumann and Maison [45] reported a low grade adenocarcinoma of the stomach in 1 of 5 rats fifteen months after injection of 3,4-benzpyrene into the submucosa of the pyloric area. A tumor, 1 by 1 cm., which had invaded the muscularis was found. There were no metastases. No further description or illustration is given. A myoma and a spindle cell sarcoma were found in 2 of the other rats.

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44. Oberling, C.; Sannie, C.; Guérin, M., and Guérin, P.: Bull. Assoc. franç. p. l'étude du cancer **25**:156, 1936.

45. Rusch, H. P.; Baumann, C. A., and Maison, G. L.: Arch. Path. **29**: 8, 1940.

(*e*) Methylcholanthrene: Van Prohaska, Brunschwig and Wilson [40] gave 15 mice 2 minims (0.12 cc.) of a 1 per cent solution of methylcholanthrene in olive oil orally three times a week. Eight mice survived for six months and were killed when they appeared weak and emaciated. Two of these had benign squamous cell papilloma of the forestomach at the end of one hundred and sixty-four and one hundred and seventy-five days, respectively. Four of the others had squamous cell carcinoma of the buccal mucosa or of the skin about the mouth. When a 1 per cent solution of methylcholanthrene in lard was injected into the mouths of 33 mice every other day for four months, no benign or malignant gastric lesion was found. One mouse had a squamous cell carcinoma of the angle of the mouth after sixty-seven days and another a hypopharyngeal squamous cell carcinoma after one hundred and forty days. These authors pointed out the close relationship of methylcholanthrene to the bile acids which normally bathe a large portion of the gastrointestinal tract. They conclude, however, that methylcholanthrene administered orally does not have a marked carcinogenic effect on the alimentary canal even in the upper portion, lined by squamous epithelium. They suggest that certain mechanical factors may explain this in part, in addition to an apparent high degree of resistance of the epithelium of the stomach and intestine. On cutaneous application and subcutaneous injection of carcinogens, prolonged contact with the cells is possible, whereas in the alimentary canal the mucosa is constantly washed by various fluids and is, moreover, covered and perhaps protected by a layer of mucus.

Necheles [46] injected 250 mg. of methylcholanthrene into the submucosa of the anterior wall of the stomach near the proximal border of the antrum in a dog. Three gastric biopsies taken in the region of the injection over a period of nine months have shown an inflammatory reaction but no evidence of malignant change. The experiment is still in progress.

Stewart [5b] reported squamous papilloma of the stomach in 4 and squamous cell carcinoma of the stomach in 4 of 30 strain A male mice which when 3 months old received injections of a solution of methylcholanthrene in mineral oil into the anterior wall of either the glandular stomach or the forestomach. All the tumors were forestomach lesions and are excellently described and illustrated. The four carcinomas were visible; one was adherent to the liver. They were located in the anterior wall except one, which was some little distance from the point of injection, at the posterior inferior margin of the forestomach. Microscopically, the infiltrating epithelium invaded all layers of the stomach and was composed of basal cells, prickle cells and flat squamous cells,

46. Necheles, H.: Personal communication to the authors.

atypical in size, shape and staining. There were numerous mitotic figures. In one tumor there were nests of epithelium within thin-walled vessels, either lymph vessels or blood vessels, between the two muscle layers of the stomach. In the case in which the abscessed liver was adherent to the wall of the stomach opposite the tumor, masses of keratin were observed within the abscess. In 2 cases small nodules composed of tumor cells were adherent to the external surface of the peritoneum. One tumor was successfully transplanted to 4 of 6 strain A mice, which had large tumors at the sites of inoculation after three weeks. Transplants of these in turn were successful in 2 of 6 mice, which had large tumors after one month. The tumors developing from the transplants were identical in morphologic characteristics to the original tumor of the stomach. In the literature on successfully induced squamous cell carcinoma of the stomach, this is one of the few reports accompanied by clear pathologic descriptions, sufficient relevant details, and photomicrographs to show the significant changes.

Stewart has indirectly raised several points of interest. Invasion of the muscularis was taken as the criterion to differentiate carcinoma from papilloma. The absence of such invasion has usually been regarded as one of the chief objections to classifying certain experimental gastric lesions as malignant. Stewart has illustrated such invasion of the muscularis and the presence of nests of epithelium within thin-walled vessels, either lymph vessels or blood vessels. The latter is usually considered a reliable indication of malignancy in spontaneous tumors. Successful transplantation of gastric tumor tissue has rarely been demonstrated before. These points will be discussed in more detail later. The possibility of coincidence is entirely eliminated by the use of pure strain mice, for no similar tumor has been observed to occur spontaneously in several thousand strain A mice.

3. Miscellaneous Factors. Twort and Bottomley [32b] reported a squamous carcinoma of the forestomach in a mouse painted with a watery solution of a mixture of chrysene ammonium and sodium sulfonite for twenty weeks. The tumor invaded the liver and adjacent organs. Since this was the only mucous membrane tumor in a large number of mice (12,000) subjected to similar procedures, it was considered to be spontaneous.

Hormonal substances have been of considerable importance in some fields of experimental induction of tumors; however, gastric lesions apparently have been rarely found. Pierson [48] reported a growth resembling mammary gland in the wall of the stomach of a castrated rabbit which had received 0.1 mg. of an estrogen twice a week for three years. In the thickened gastric wall were numerous infiltrating tubules

47. Footnote deleted.

48. Pierson, H.: Ztschr. f. Krebsforsch. **48**:177, 1938.

and ducts filled with a material staining pink with eosin. This glandular tissue was nowhere seen to be related to the gastric epithelium in serial sections. There was extensive squamous cell metaplasia. This was the only such tumor found in a large group of rabbits receiving the estrogen.

Domagk [49] reproted a gastric adenocarcinoma in 1 of 20 mice fed a diet of rice and 20 per cent olive oil, alternating each week with the usual diet, for one year. The tumor was visible grossly. Microscopically, it consisted of atypical glandular epithelium with numerous mitotic figures and extended through the muscularis mucosae and the muscularis but was limited by the serosa. There were associated inflammatory changes in the gastric wall. No metastasis is mentioned. Photomicrographs of this tumor show the changes described. Gastric polyposis, regarded as precancerous, was found in some of the other mice. A pulmonary nodule in 1 mouse was interpreted to be a metastasis from the stomach, although the gastric lesion was apparently considered precancerous. There is no photograph or description of the pulmonary nodule. No similar gastric lesion was found in 20 mice that were not fed olive oil.

COMMENT

Nomenclature.—The term "experimental gastric carcinoma" almost always refers to squamous cell carcinoma of the forestomach. In a sense this is confusing, since carcinoma of the stomach in man implies adenocarcinoma. There is no experimental evidence to suggest that the two types are related except in the anatomic location of both in the stomach. Occasionally the term "experimental gastric carcinoma" has been applied to malignant tumors of other organs transplanted to the gastric wall. The use of the phrase in this sense is misleading.[50]

Sarcoma.—Reports of experimental sarcoma of the stomach are uncommon and have not been included in this review. Brunschwig [51] found large fibrosarcomas in 2 of 3 male rats exposed for an indeterminite length of time to various carcinogenic agents. Each of these animals also had a benign fibroadenoma of the breast.

Adenocarcinoma.—There are in the literature relatively few claims [52] of the production of experimental adenocarcinoma of the stomach. On close scrutiny these reports either are not convincing or do not permit objective evaluation of the malignant nature of the changes. There is no well established case of adenocarcinoma of the stomach resulting from an experimental procedure.

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49. Domagk, G.: Ztschr. f. Krebsforsch. **48**:283, 1938.

50. Besredka, A., and Gross, L.: Ann. Inst. Pasteur **62**:253, 1939; abstracted, Am. J. Cancer **37**:124, 1939.

51. Brunschwig, A.: Personal communication to the authors.

52. Voronoff and Alexandrescu.[30] Roffo.[42] Waterman.[43] Rusch and others.[45] Domagk.[49]

The fact that attempts to induce adenocarcinoma of the stomach with known carcinogenic agents have been unsuccessful raises several considerations.

1. Time Factor. The induction period may be considerably longer than for other experimental cancers.

2. Mechanical Factors. Van Prohaska, Brunschwig and Wilson [40] called attention to the problem of keeping the carcinogenic agent in the stomach and, even when this is accomplished, of bringing the agent into intimate contact with the secreting gastric mucosa covered with mucus. The implantation or injection of carcinogenic compounds into the wall of the glandular stomach eliminates some of the mechanical difficulties, but these methods have not yet yielded the positive results that might be expected. Although these procedures may be of considerable value, particularly in the selection of effective carcinogens, feeding experiments perhaps have the advantage of being more physiologic.

3. Chemical Factors. Perhaps there is a carcinogenic agent, specific for the glandular tissue of the stomach. Although this is purely speculative, there is some basis for such a possibility. Shear's [53] studies in pure strain mice indicate that 2-amino-5-azotoluene is a specific carcinogenic compound for liver tissue. Methylcholanthrene, which is closely related to bile acids, appeared on theoretic grounds to be possibly carcinogenic for gastric glandular tissue. Reports, however, do not yet indicate that it has such an action when given by mouth (Van Prohaska, Brunschwig and Wilson [40]) or when injected into the gastric wall (Stewart [5b]).

4. Species or Strain Susceptibility. Experimental cancer may at times be induced in an organ or tissue of an animal while the same procedure in other animals of a closely related species or strain has no carcinogenic effect. The well known difference in the response of rats and rabbits to tarring is a classic example. Relatively few species have been used in attempts to induce gastric adenocarcinoma. The possibility that a species or strain susceptible to known carcinogenic agents may exist is not entirely excluded.

5. Tissue Resistance. A more basic factor may be the apparent resistance of normal gastric glandular tissue to a carcinomatous change. There is considerable pathologic evidence that carcinoma does not develop in an unaltered gastric mucosa (Konjetzny [7]). The question arises as to whether the known carcinogenic agents can be effective in the normal glandular stomach of an animal. Possibly preliminary benign changes are prerequisite. The more important question would then still remain as to what brings about the earlier benign changes.

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53. Shear, M. J.: Am. J. Cancer **29**:269, 1937.

Squamous Cell Carcinoma.—It is generally accepted that squamous cell carcinoma of the forestomach has been experimentally produced in rats and mice. However, the impression given at times that it has been produced rather commonly is not supported by a review of the literature. The actual number of instances on record is difficult to estimate in view of the doubtful malignancy of some induced forestomach tumors regarded as carcinoma and the questionable relation of some of these to the experimental procedure. In this regard Stewart's [5b] work gives promise of more successful and consistent results in the near future. One of his observations is particularly noteworthy: With methylcholanthrene the estimated average induction period of both benign and malignant squamous cell tumors of the stomach was about fourteen months. This is a considerably longer period than is needed to induce tumors of other tissues with methylcholanthrene in the same strain of mice. Although this apparently greater resistance of gastric squamous epithelium to carcinogenic action is not definitely known to exist in all animals and for all carcinogenic compounds, the numerous negative results obtained in experiments of shorter duration strongly suggest this possibility.

In weighing the evidence of malignancy, the presence of a mass of atypical or keratinized squamous cell tissue or of a large cavity filled with caseous material in the lung of rats usually does not indicate a metastasis. It is so frequently found that not much significance can be attached to it. Bullock and Rohdenburg [16] called attention to this fact in 1918 and in an accompanying photomicrograph showed squamous cell epithelium with mitotic figures in the lung of a rat with bronchopneumonia. Bonne [9b] later found a large keratinized squamous mass in the periphery of the lung of a rat dying of bronchopneumonia. No connection with a bronchus could be found. There was no squamous cell tumor elsewhere, and it was interpreted to be a metaplastic mass primary in the lung. Wolbach and Howe [21] and others found widespread squamous cell metaplasia in vitamin A–deficient rats. None, however, have shown the presence of squamous cell metaplasia and bronchiectasis in rats as convincingly as Passey, Leese and Knox.[13c] These workers found that 51 per cent of a large group of laboratory rats had bronchiectasis in some degree or other, which occurred independent of the diet. Some bronchiectatic cavities filled with purulent material replaced several lobes of the lung. Squamous cell metaplasia of the bronchial tree was also common and independent of the diet, although it was more marked in vitamin A–deficient rats. Keratinization of these metaplastic masses was almost exclusively limited to the vitamin A–deficient animals. In 1 rat and 2 mice squamous cell metaplasia of the alveoli was found. These lesions are excellently illustrated. There

is no photomicrograph in the literature of a so-called metastasis to the lung from a squamous cell tumor of the forestomach that cannot be matched among those accompanying the paper of Passey, Leese and Knox. These authors considered the possibility of several etiologic factors without reaching a definite conclusion. It is certain, however, the lesions are not metastases. This is confirmed by many unpublished observations (Steiner [54]).

Abdominal metastases have apparently not been so confusing. Enlarged abdominal lymph nodes obviously do not indicate metastasis in all cases. In the presence of severely infected benign forestomach changes, it might be surprising if the regional lymph glands were not enlarged. For this reason illustrations of such enlargements are not convincing unless accompanied by photomicrographs showing the metastatic tissue. Likewise there are causes other than carcinomatous invasion of neighboring structures to account for adhesions between the stomach and these organs.

Evidence in favor of malignancy could be shown by irreversibility of experimental gastric lesions simulating carcinoma. Obviously it is not always possible to demonstrate this, especially when the carcinogenic agents are injected into the gastric wall. In the case of dietary deficiencies or the feeding of carcinogenic agents this method seems applicable. Bonne's [9b] report of a squamous cell carcinoma in a mouse which had received tar by mouth for thirty-three weeks and which died almost five months later shows that the method is feasible. Another example is the experiment of Hoelzel and DaCosta [23b] in which they used dietary means to induce forestomach lesions in rats, which almost disappeared when a complete diet was resumed.

Transplantation of squamous cell tumors of the forestomach has not been attended by much success. This may have been due to (1) infection of the implants, as Fibiger and others have emphasized, (2) the nonmalignant nature of some of the tumors, (3) the large amount of acellular and keratinized material present or (4) the difficulties in transplanting tissue from one strain of animals to another. Fibiger [14c] apparently succeeded in the case of a mouse. Metastases to lymph nodes were transplanted into 28 of 55 mice that survived inoculation, representing four generations of "takes" covering a period of one year. The transplanted tissue maintained the histologic appearance of the original tumor of the stomach. Slye, Holmes and Wells [4] in the case of two spontaneous gastric carcinomas in mice obtained only a few takes with one of the tumors, which could not be carried to a second generation. Stewart [5b] transplanted an induced squamous cell tumor of the stomach of a mouse through two generations. Microscopically, the transplants were identical to the original tumor.

54. Steiner, P. E.: Personal communication to the authors.

In this connection the experience with transplantation of induced squamous cell tumors of the skin deserves consideration. Woglom [55] in a comprehensive review of experimental tar cancer stated that spontaneous keratinizing neoplasms had long been known to be refractory to propagation and that similar difficulties were encountered with induced squamous cell tumors even when they gave definite evidence of being malignant in other ways. The failure to obtain "takes" with such tumors cannot, therefore, be regarded as eliminating the possibility of malignancy. On the other hand, a positive result might be due merely to temporary proliferation of the grafts. In either case the result might be equivocal. Murray and Woglom (cited by Woglom [55]) found autotransplants in mice more satisfactory than homotransplants. Normal tissues or benign growths proliferated only temporarily or not at all, whereas malignant growths gave a high percentage of positive results. In the hands of these observers autotransplantation was sometimes successful with tar tumors that appeared histologically benign at the time of implantation. This was regarded as evidence that autotransplantation could at times facilitate the recognition of a malignant tumor before histologic examination. Kreyberg [56] was unable to confirm the observation that tumors appearing histologically benign in mice were autotransplantable.

Rous and Kidd [57] noted that autotransplants of a virus papilloma in rabbits in some instances grew while the original papilloma retrogressed. This was found particularly when inflammation of bacterial origin ensued at the site of inoculation.

These examples illustrate the difficulties encountered in interpreting the significance of both successful and unsuccessful transplants of induced squamous cell tumors of the skin. Since these experimental tumors are the ones most analogous to those of the forestomach, the same uncertain interpretation may apply to the results of transplantation of the latter as well.

Criteria of Malignancy.—The question of what characterizes an experimental carcinoma has been a source of controversy. Fibiger [14c] set down clearly his criteria of malignancy with respect to squamous cell tumors of the forestomach and supported his opinion by pointing out the similarity of such changes to those occurring in spontaneous squamous cell carcinoma in man and animals. These criteria were:

1. Heterotopic downgrowth of epithelial cells, not only the normal type of the basal epithelial layers, but, mixed with these, atypical and keratinized cells in abundance, partly arranged as spherical masses and horny globes.

55. Woglom, W. H.: Arch. Path. **2**:533 and 709, 1926.
56. Kreyberg, L.: J. Cancer Research **9**:381, 1925.
57. Rous, P., and Kidd, J. G.: J. Exper. Med. **69**:399, 1939.

2. Infiltrative growth of these heterotopic and atypical epithelial cells into the deeper layers, splitting up invasively the elements of the connective tissue of the mucosa and the muscle cells of the muscularis mucosae, forming isles and spurs in the latter—as most frequently seen—also penetrating through this membrane into the superficial or deeper layers of the submucosa.

Bullock and Rohdenburg [16] questioned the malignant nature of Spiroptera tumors such as Fibiger's in the absence of active invasion of the muscularis and definite metastases. Bonne placed considerable reliance on invasion of blood vessels as a sign of malignancy in 1 case [9a] and infiltration of the muscularis in another.[9b] Stewart [5b] differentiated papilloma from carcinoma on the basis of invasion of the outer muscular layers of the stomach. Additional evidence of malignancy was also present in one case in which nests of epithelium were found in thin-walled vessels between the muscle layers of the stomach, either lymph vessels or blood vessels; and in another the tumor was successfully transplanted to other mice through two generations.

A fundamental question is whether the microscopic criteria of spontaneous malignancy can be applied to experimental tumors in differentiating the malignant from the benign. In the final analysis, malignancy may be judged only by the course of events. Certain histologic characteristics of spontaneous lesions have become synonymous with malignancy; however, this relationship was at first empirically established. Tumors following a malignant course were found to have some differentiating morphologic characteristics. Earlier and earlier stages of these characteristics were recognized, so that now it is often possible to predict that a spontaneous tumor will follow a malignant course on the basis of its structure only and without any definite proof of malignant activity beyond a few microscopic changes which, if they did not progress, could not be classified as malignant and would not interfere with the well-being of the organism. If early morphologic changes from the accepted normal are used to predict a malignant sequence, they are reliable guides only so far as this sequence has been shown always to follow. It cannot be inferred with certainty that the histologic criteria established empirically as pointing to an irreversible and progressive process in a spontaneous tumor necessarily apply to induced tumors or other forms of spontaneous tumors. If a malignant alteration represents an intrinsic irreversible cellular change, manifested by aggressive and destructive invasion of other tissues and organs and continuing until the death of the organism, some of the criteria of malignancy applied to induced tumors may at times be inadequate.

These remarks in relation to experimental squamous cell carcinoma of the forestomach can become the subject of futile controversy. However, if detailed and careful studies of induced squamous cell tumors

of the skin, such as those of Yamagiwa and Ichikawa [58] and Ichikawa and Baum [59] and the comprehensive reviews of Woglom [55] and Seelig and Cooper,[60] are again consulted, some light may be thrown on the problem.

Murray and Woglom (cited by Woglom [55]) made use of four criteria of malignancy: "(1) the progressive growth of a tumor after painting has been discontinued, and the growth of its autotransplants; (2) the recurrence after wide excision; (3) histologic evidence of local infiltrative growth, and (4) metastases. They regarded the loss of differentiation and the presence of atypical cellular characteristics as wholly inadequate." These investigators apparently found that simpler criteria of induced malignancy did not distinguish benign from malignant tumors.

Rous and Kidd [57] described squamous cell tumors in rabbits following repeated tarring of the ear which have all the morphologic appearances of carcinoma, including extension through lacunae in the cartilage and presence of proliferating epithelium in the lymphatics. These tumors were at times morphologically indistinguishable from carcinoma and were called carcinoids. When the tarring was discontinued, the carcinoids retrogressed, whereas the carcinomas continued to show active invasive properties. If the tarring was continued without interruption, the carcinoids eventually ceased to grow destructively and took on the gross forms of old papillomas. These investigators also mentioned a group of tumors which had active malignant properties only as long as tarring was continued.

On the basis of these and numerous similar observations in regard to experimental squamous cell tumors of the skin, it is questionable whether the morphologic characteristics of spontaneous squamous cell carcinoma in man or animals can be applied to induced gastric tumors unless *the latter continue to show malignant activities in the absence of the agent used to bring them about.* Fibiger believed he demonstrated such an irreversible change, but the factor of a dietary deficiency was not eliminated in his experiments. Since some dietary deficiencies in themselves apparently lead to forestomach changes meeting Fibiger's criteria of malignancy, the validity of his interpretation is open to question. In view of this there is obviously some hesitancy in agreeing that all experimental squamous cell tumors of the forestomach regarded in the literature as carcinoma do actually represent malignant alteration of tissues.

What criteria would serve to differentiate induced benign and malignant gastric tumors cannot be settled at present. More of these tumors

58. Yamagiwa, K., and Ichikawa, K.: J. Cancer Research **3**:1, 1918.
59. Ichikawa, K., and Baum, S. M.: J. Cancer Research **9**:85, 1925.
60. Seelig, M. G., and Cooper, Z. K.: Am. J. Cancer **17**:589, 1933.

will have to be observed carefully in order to determine their own peculiarities. It is not known, for example, whether such carcinomas are more apt to metastasize than they are to invade neighboring organs; or whether invasion of the muscularis indicates a definitely irreversible process. Induced cancers should, however, have those characteristics generally considered inherent in a malignant growth: (1) the ability to proliferate independently as metastases; (2) the ability to invade progressively and destructively neighboring tissues and organs; (3) irreversibility of these properties in the absence of the extrinsic factor initially held responsible for the cellular change. Since such changes have been observed to occur in spontaneous squamous cell carcinoma of the forestomach in mice (Slye, Holmes and Wells;[4] Wells, Slye and Holmes[8]) there should be, in addition, (4) reasonable evidence to indicate a causal relation of the experimental procedure to the tumor.

Application of these criteria to induced gastric tumors is not always easy, but unless they can be demonstrated, the malignant nature of the tumor cannot be considered as proved. The histologic appearance of the tumor alone is not adequate.

SUMMARY

Spontaneous gastric adenocarcinoma is found rarely in animals and for all practical purposes can be said to occur exclusively in man. Consequently studies of this most important human malignant lesion are limited for the present to clinical material. A review of the literature discloses no reliable method of inducing adenocarcinoma of the stomach in animals; in fact, there is no well established case of an adenocarcinoma of the stomach produced experimentally.

Some success has attended efforts to induce squamous cell carcinoma of the forestomach in mice and rats, although considerably less than the claims in the literature would indicate. There is no experimental evidence to suggest that the two types of gastric carcinoma are related except in the anatomic location of both in the stomach.

The criteria of induced malignancy are considered. Proof of malignancy is not given by the histologic appearance of a tumor, whether spontaneous or induced, but by the demonstration of malignant activity as evidenced by: (1) the ability to proliferate independently as metastases; (2) the ability to invade progressively and destructively neighboring tissues and organs; (3) irreversibility of these activities, which must be shown to continue in the absence of the extrinsic factor initially held responsible for the cellular changes. It must, furthermore, be shown that (4) a given malignant change occurs with sufficient regularity to establish a causal relationship of the experimental procedure to the cancer.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Alfred H. W. Caulfeild, research member of the Connaught Laboratories, Toronto, Canada, died on May 2, at the age of 59.

Esmond R. Long, Philadelphia, has been elected a member of the American Philosophical Society.

Valy Menkin has been advanced to the rank of assistant professor of pathology at the Harvard Medical School.

The Rockefeller Foundation has made a grant of $15,000 toward the development over a three year period of legal medicine at Harvard Medical School.

The George M. Kober Medal of the Association of American Physicians for 1940 has been presented to F. F. Russell for his introduction of double sugar medium for the cultivation of typhoid bacilli, and the same medal for 1941 has been awarded to William deB. MacNider for his study of acquired resistance of tissue cells after injury to the liver and kidney.

The old autopsy house of the Philadelphia General Hospital ("Old Blockley"), which has been restored and named the Osler Memorial Building, was dedicated on June 8. At the same time the painting "Osler at Old Blockley" by Dean Cornwell was shown.

Louis Hamman, Johns Hopkins University, has been elected president of the Association of American Physicians.

The Ettore Marchiafava Prize, founded by the University of Rome in 1938 for the best work on morbid anatomy or general pathology, has been awarded to Mario Monacelli, director of the dermatologic clinic of the University of Messina, and Giulio Raffaele, of the University of Rome.

Society News.—The Biologic Photographic Association will meet at the Hotel Schroeder, Milwaukee, Sept. 12 to 14, 1940. The address of the secretary is Magee Hospital, Pittsburgh.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Pathologic Chemistry and Physics

Increasing Plasma Prothrombin in the Newborn Infant. L. B. Shettles, E. Delfs and L. M. Hellman, Bull. Johns Hopkins Hosp. **65**:419, 1939.

The plasma prothrombin level of the newborn infant can be raised not only by feeding vitamin K concentrate directly to the infant after birth but also by administering it to the mother prior to delivery. The values obtained by administration of the concentrate to the mother are often three times those normally seen and in general are higher than those which can be attained by administering vitamin K concentrate to the baby after birth. From Authors' Summary.

An Electrophoretic Study of Nephrotic Serum and Urine. L. G. Longsworth and D. A. MacInnes, J. Exper. Med. **71**:77, 1940.

The electrophoretic patterns of the serum and urine of 2 patients with lipoid nephrosis have been obtained and have been compared with a typical pattern of normal serum. The patterns of the pathologic serums deviated widely from the normal; albumin was relatively low and globulin high. The comparison of the patterns of nephrotic serums cleared by centrifugation and by ether extraction shows that a large portion of the beta globulin consisted of a labile lipoprotein. The pattern of the proteins of the nephrotic urine resembled that of the proteins of normal serum, with, however, significant differences.

From Authors' Summary.

Vitamin K. E. A. Doisy and others, Science **91**:58, 1940.

During the decade following Dam's first observations on the hemorrhagic syndrome the combined efforts of several groups of investigators have solved many of the important problems connected with the new vitamin. Sources of vitamin K were discovered, methods of extraction and purification devised, the isolation effected, the structure of K_1 worked out and then verified by synthesis, and a promising start made on the therapeutic applications. In addition, simple water-soluble compounds with antihemorrhagic properties have been supplied for clinical work. Preliminary results with these compounds are encouraging.

From Authors' Summary.

Adrenalin Content of the Adrenal Glands in Diphtheria Intoxication. C. A. Ashford, Brit. J. Exper. Path. **20**:385, 1939.

Ashford has estimated the adrenalin content of the adrenal glands in diphtheria intoxication to determine whether the content is reduced during the acute stages of diphtheritic toxemia. Reports on this point, based mainly on histologic methods applied to experimental animals and human postmortem material, have been conflicting and a number of theories of the circulatory collapse have been built up on the assumption of a depletion or an exhaustion of the medullary adrenalin. Guinea pigs have been used, and the results show that reduction of adrenalin content to any marked extent is not found. Histologic observations are reported briefly, and the possible significance of cortical damage discussed.

Microbiology and Parasitology

TOXOPLASMIC ENCEPHALOMYELITIS. A. WOLF, D. COWAN and B. H. PAIGE, Am. J. Path. **15**:657, 1939.

A fifth case of a new disease, granulomatous encephalomyelitis due to a protozoon, occurring in an infant is described. The clinical and pathologic observations in this case are shown to be similar to those in the first 4 cases. This group represents a distinct disease entity. The disease affects young infants, produces manifestations of general involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the eyegrounds and terminates fatally after an acute or subacute course. The spinal fluid shows xanthochromia, a high protein content and pleocytosis. The central nervous system is the site of focal inflammatory and degenerative lesions, which are widely disseminated. Similar changes are found in the retina and choroid. Miliary granulomas are a characteristic feature of the process in the nervous system. Focal inflammatory lesions were present in the heart and striated muscle in 1 case. A protozoan parasite is present in all the lesions. The results of transmission of the infection to animals from the case reported here indicate that the causative protozoon is a Toxoplasma. The designation Toxoplasma hominis is suggested for the microorganism and the term "toxoplasmic encephalomyelitis" for the disease.

FROM AUTHORS' SUMMARY.

BACTERIOSTATIC AND ANTITOXIC ACTIONS OF SULFANILAMIDE. JOSEPH T. KING, AUSTIN F. HENSCHEL and BERYL S. GREEN, J. A. M. A. **113**:1704, 1939.

A tissue culture study has been made of the bacteriostatic and antihemolytic properties of sulfanilamide. All strains of beta streptococci studied were inhibited. The bacteriostatic effect varied directly with the concentration of the drug and inversely with the number of bacterial colonies. Reduction in hemolysis was observed to accompany bacteriostasis. Correlation of the reduction in hemolysis with the observed bacteriostasis leads to the conclusion that the antihemolytic effect is secondary to bacteriostasis. The drug regularly inhibits the wide, diffuse peripheries usually seen around colonies of beta streptococci growing in clots of tissue culture. The drug causes the development of abnormal, long chains of streptococci.

FROM AUTHORS' SUMMARY.

HEMOLYTIC STREPTOCOCCUS LYMPHADENITIS IN GUINEA PIGS. C. V. SEASTONE, J. Exper. Med. **70**:347, 1939.

A group of guinea pigs carrying a chronic streptococcic cervical lymphadenitis has been studied. The chronic disease may be transmitted with pure cultures of streptococci isolated from the naturally occurring abscesses. Its probable mode of transmission under natural conditions was shown to be by ingestion of the infective agent. The spontaneous appearance of an acutely fatal variant was observed. Infection with the chronic strains protected animals against the highly virulent strain. Such immunity could not be passively transferred to either mice or guinea pigs, nor could any opsonizing, precipitating or bactericidal antibody be associated with it. Allergy could not be correlated with this immunity. The dissociation of the chronic and acute strains was investigated, and organisms in noninvasive phases were isolated. No precipitin reaction attributable to an antigenic virulence factor could be demonstrated. No protection was obtained with vaccines or aggressins.

FROM AUTHOR'S SUMMARY.

VIRULENCE OF GROUP C HEMOLYTIC STREPTOCOCCI. C. V. SEASTONE, J. Exper. Med. **70**:361, 1939.

A nonantigenic mucoid polysaccharide similar to that described by Kendall, Heidelberger and Dawson was isolated from group C hemolytic streptococci.

A simple method for its quantitative estimation is described. By means of this method, as well as by the direct isolation of the carbohydrates, the size and persistence of capsules in young cultures of various strains have been related to the nonantigenic mucoid polysaccharide.

PURITY OF PREPARATIONS OF ELEMENTARY BODIES OF VACCINIA. J. E. SMADEL, T. M. RIVERS and E. G. PICKELS, J. Exper. Med. **70**:379, 1939.

A method of estimating the purity of preparations of elementary bodies of vaccinia is described. It depends on the comparison of the number of infective units of virus in a given material with the number of elementary bodies. The latter figure is estimated from the dry weight of the preparation by means of a calculated value for the weight of a single dehydrated elementary body. The ratio of the number of infective units of vaccine virus to the number of elementary bodies varied between 1:2.4 and 1:9.2 in seven consecutive experiments; the average was 1:4.2. These ratios indicate a high degree of purity of the preparation. Moreover, they indicate that a relatively high percentage of the elementary bodies in the preparations was infective.

FROM AUTHORS' SUMMARY.

MALIGNANT PANLEUKOPENIA OF CATS. W. D. HAMMON and J. F. ENDERS, J. Exper. Med. **70**:557, 1939.

The most conspicuous clinical finding in the course of this virus disease is fulminating panleukopenia. The earliest significant decrease noted is usually in the number of the lymphocytes. From the study of lymph nodes and bone marrow during the period of incubation and throughout the illness, it appears that a failure of leukopoiesis is the cause of the leukopenia. Inclusion bodies in the primitive blood cells of the marrow suggest a direct action of the virus on these cells. When recovery occurs, a marked myelogenous leukemoid response is noted. Available data indicate the presence of mild anemia due to a failure in erythropoiesis, less marked than the leukopenia, probably because of the longer life of the adult circulating erythrocytes. The erythrocytes appear to have increased fragility, and the serum has a slight increase in icterus, suggesting an increased mean erythrocyte age. During recovery erythropoiesis does not begin until after the myeloid marrow response has begun to subside, possibly because of previous mechanical crowding of the marrow by the more rapidly growing myeloblasts and myelocytes.

FROM AUTHORS' SUMMARY.

INCLUSION BODIES IN SCARLET FEVER. J. BROADHURST and others, J. Infect. Dis. **64**:193, 1939.

That a virus is concerned in the production of scarlet fever is indicated by: the presence of inclusion bodies in the tissues of the upper respiratory area of patients with scarlet fever; the presence of inclusion bodies in the white blood corpuscles of such patients; the presence of inclusion bodies in serial tissue cultures inoculated with bacteria-free filtrates of blood from such patients.

FROM AUTHORS' CONCLUSIONS.

BILE ACTION ON PNEUMOCOCCI AND STREPTOCOCCI. P. H. GREEY, J. Infect. Dis. **64**:206, 1939.

Pneumococcus and Streptococcus viridans on blood agar alter the red cells surrounding their colonies so that these cells are resistant to the hemolytic effect of bile. Dried bile placed on the blood agar colonies of Pneumococcus dissolves the colonies but leaves the zone of fixed cells intact. Colonies of Str. viridans and Streptococcus haemolyticus are not dissolved. This is therefore a simple means of differentiating these colonies.

FROM AUTHOR'S SUMMARY.

VIABILITY OF TUBERCLE BACILLI ON RESTRICTION OF OXYGEN. T. S. POTTER, J. Infect. Dis. **64**:261, 1939.

Human tubercle bacilli, like avian, may survive prolonged drastic deprival of oxygen at 37 C. Avian bacilli, like the human type, studied by Novy and Soule, are unable to survive a relatively mild restriction of oxygen at 37 C. when such restriction is combined with an accumulation of the gaseous products of their metabolism on glycerin agar. FROM AUTHOR'S CONCLUSIONS.

THE NUTRITION OF CORYNEBACTERIUM DIPHTHERIAE. W. C. EVANS, W. R. C. HANDLEY and F. C. HAPPOLD, Brit. J. Exper. Path. **20**:396, 1939.

Evans, Handley and Happold, continuing their studies on the nutritional requirements of Corynebacterium diphtheriae types, find that the additional growth factor present in tissue extractives required by certain gravis strains is pantothenic acid. The mitis and some gravis strains which had previously been grown in chemically defined mediums synthesize this "gravis growth factor." Pantothenic acid is a compound of beta-alanine and a dihydroxyvaleric acid; according to current literature, this substance is identical with the chick antidermatitis factor and is one of the B complex vitamins. The paper also describes experiments which show that strains of C. diphtheriae grown in chemically defined mediums synthesize substances with physiologic activities similar to those of aneurin, riboflavin and "co-enzymes I or II."

INFLUENZAL BRONCHITIS. M. STRAUB, J. Path. & Bact. **50**:31, 1940.

In mice the virus of influenza affects specifically the epithelium of the respiratory tract from the bronchioles to the bifurcation of the trachea. It causes catarrhal bronchitis with collapse of lung tissue. This collapse is merely a complication of the epithelial process and is not in itself specific. It may be absent if a weak virus has been used or if the mice are adults. In such circumstances treatment with diluted diphtheria toxin prior to infection or treatment with Tyrode solution one or two days after infection is capable of aggravating the consequences of the diffuse catarrhal inflammation. In influenzal bronchitis, after a degenerative stage there follows a regenerative stage of the epithelial process. In the larger bronchi, this entails restitutio ad integrum: in the bronchioles, metaplastic epithelial changes may occur. Immunity to influenza virus in mice depends largely on the presence of such epithelialized areas. Even very slight metaplastic change seems to give complete protection. FROM AUTHOR'S SUMMARY.

HISTOPHYSIOLOGY OF THE TUBERCLE. E. TONUTTI and J. WALLRAFF, Beitr. z. path. Anat. u. z. allg. Path. **103**:78, 1939.

A series of rabbits were infected by the technic of Bieling and Schwartz. They received a preliminary sensitizing injection of killed human tubercle bacilli and three weeks later an intravenous injection of living tubercle bacilli of the bovine type. During the entire duration of the experiments the animals were maintained on a normal diet supplemented with vitamin C. The histiocytes and epithelioid cells in the tuberculous lesions situated in the lungs, adrenals and elsewhere were found to contain a striking amount of ascorbic acid by the silver nitrate technic, as well as phagocytosed tubercle bacilli. In sharp contrast, the lymphocytes contained no histochemically demonstrable vitamin C. R. J. LEBOWICH.

Immunology

THE SITE OF SENSITIVITY IN THE ARTHUS PHENOMENON. A. R. RICH and R. H. FOLLIS JR., Bull. Johns Hopkins Hosp. **66**:106, 1940.

In the Arthus type of hypersensitivity there is vascular sensitivity, but the cells of the tissues at large are not sensitized. Tissue death in the Arthus reaction

results primarily from impairment of nutrition due to vascular damage and to clogging of the tissue spaces with exudate and hemorrhage. It has been shown by numerous investigators that if foreign protein is injected one or more times into the cornea of one eye a subsequent injection after some days leads to a more marked corneal reaction than occurs as a result of an injection of the protein into the opposite, untreated eye. This has been attributed to the development of local hypersensitivity in the treated eye. The present experiments indicate that the more marked reaction in the treated eye is due not to a higher degree of local sensitization of the corneal tissue but to the increased vascularity which results from the preliminary intracorneal injections, from which a greater amount of exudate can be derived more promptly.

FROM AUTHORS' SUMMARY.

SEROLOGIC STUDIES ON SUGAR. J. M. NEILL and others, J. Exper. Med. **70**:427, 1939.

Solutions of all of the chemically pure sucrose reagents of American manufacture that were tested gave reactions with type II antipneumococcus serum. The capacity of that antiserum to react with sucrose solutions was removed by absorption with the homologous pneumococci or with Leuconostoc mesenterioides. The serologic reactivity was due not to sucrose itself but to accompanying substances that could be removed by precipitation with a proper concentration of alcohol or by treatment with activated carbon. Although type II antipneumococcus serum was used to detect its presence, the reactive material found in the sucrose can be considered to be only related to, and not identical with, antigens of type II pneumococci.

FROM AUTHORS' SUMMARY.

BACTERIAL AGGLUTINATION. H. E. ALEXANDER and M. HEIDELBERGER, J. Exper. Med. **71**:1, 1940.

The quantitative, absolute methods of agglutinin and precipitin analysis previously developed for antipneumococcus serum have been shown to be applicable to horse and rabbit serum and plasma containing antibody for influenza virus type B. With the aid of these methods and improved immunization schedules, the antibody content of the rabbit serum has been increased five to ten times. The method recommended for the purification of rabbit antipneumococcus antibody has also been found applicable to the antibody for influenza virus type B in rabbit serum.

FROM AUTHORS' SUMMARY.

PERMEABILITY OF THE HUMAN PLACENTA TO ANTIBODIES. A. S. WIENER and I. J. SILVERMAN, J. Exper. Med. **71**:21, 1940.

The ratio of the titer of any of the various antibodies, e. g., hemagglutinins and syphilitic reagin, in the maternal blood to that of the corresponding antibody in the umbilical cord blood was found to be relatively constant, the value falling somewhere between 8 and 16. This figure may be considered the "index of permeability" of human placenta to antibodies, or the coefficient of the distribution of antibodies between maternal and cord blood. The possible application of these findings to the study of placental permeability to sensitizing antibodies (or reagins) is discussed.

FROM AUTHORS' SUMMARY.

ANTIGENIC RELATIONSHIPS OF STRAINS OF BACTERIUM NECROPHORUM. P. H. WALKER and G. M. DACK, J. Infect. Dis. **64**:285, 1939.

A serologic study was made of 12 strains of Bacterium necrophorum—9 of human and 3 of bovine origin. Rabbits were immunized with 10 different strains of Bact. necrophorum. The immune serum thus produced agglutinated homologous strains in dilutions ranging from 1:160 to 1:1,280. Some heterologous strains were agglutinated to titer or nearly so by certain of these antiserums.

Distinct antigenic groups were demonstrated by means of agglutination and agglutinin absorption tests. One comprised 3 strains, another 2 and a third 3. Four other strains were highly specific and probably represented other serologic groups. Virulence of these strains, as evidenced by their pathogenicity for rabbits, was no criterion for differentiating antigenic relationships, since 2 virulent strains were antigenically related to a strain of low virulence. Source of strains was not a differentiating characteristic, for of strains from chronic ulcerative colitis one was serologically related to a strain isolated from a metastatic subacromial abscess, and another, to a strain from the blood stream.

TESTS OF ANTIPNEUMOCOCCIC TREATMENTS IN RATS. W. J. NUNGESTER and A. H. KEMPF, J. Infect. Dis. **64**:288, 1939.

It is possible to study the effects of various therapeutic procedures for pneumonia in rats with experimentally produced pneumonic lesions. Such lesions parallel more closely the conditions encountered in man than do infections in rabbits or mice, which are primarily involvements of the blood stream with limited nonpulmonary lesions. In the rat the difficulty of favorably influencing the course of the disease was readily demonstrated. Specific immune serum decreased the incidence and mortality of pleurisy. However, a very appreciable mortality remained despite treatment.

FROM AUTHORS' SUMMARY.

SEROLOGIC IDENTIFICATION OF CLOSTRIDIUM TETANI. J. D. MACLENNAN, Brit. J. Exper. Path. **20**:371, 1939.

In his study of the agglutination reactions of Clostridium tetani Maclennan confirmed and extended the observations of Gunnison. He suggests the use of O antiserum as an aid to the recognition of aberrant strains. By this means he has identified a new serologic type of Cl. tetani.

PASSIVE IMMUNIZATION TO THE VIRUS OF INFLUENZA. R. HARE, J. Path. & Bact. **49**:411, 1939.

It is possible to protect mice against intranasal infection with influenza virus by prior intraperitoneal administration of ferret immune serum. When the serum is administered after the virus, its effect depends on (*a*) the time after infection at which it is given and (*b*) the severity of the infection (number of minimal lethal doses of virus inoculated). The immune serum obtained on infection with the PR 8 strain of virus is effective against heterologous strains in passive immunity experiments. The serum of a horse immunized with influenza virus was less effective in passive immunity experiments than ferret immune serum, even in the case of concentrates which in neutralizing activity were comparable to the ferret serum.

FROM AUTHOR'S CONCLUSIONS.

A STUDY OF HEMORRHAGIC PHENOMENA. A. ALECHINSKY, Ann. Inst. Pasteur **63**:41, 1939.

The Sanarelli and the Shwartzman phenomena were produced simultaneously in rabbits by a special technic and were shown to be identical. This hemorrhagic phenomenon has nothing in common with anaphylaxis. The reactions could not be elicited by horse serum, and passive transfer could not be demonstrated. The active substances which induced these reactions were found in living and dead cultures and in filtrates of various bacteria and seemed to be associated with toxins. Detailed histologic studies of the lesions produced in the Sanarelli-Shwartzman phenomena were made. The vascular changes on which the reaction depends were produced after the primary injection, but vascular rupture and thrombotic and necrotic lesions of the liver, kidneys and lungs occurred only after the second, or shocking, dose. The preparatory injection caused alterations in the hepatic

lobules and in the walls of the glomeruli, but the development of hemorrhagic and necrotic areas was uniquely connected with the shocking dose. True rupture of the endothelium of the capillaries occurred in the lung. M. P. LUXEN.

EFFECT OF BACTERIA ON SERUMS GIVING NEGATIVE WASSERMANN REACTIONS. O. SIEVERS, Acta. path. et microbiol. Scandinav. **16**:365, 1939.

Serums giving negative Wassermann reactions gave positive reactions from one to two days and anticomplementary reactions six days after inoculation with Bacillus cereus. The change became manifest earlier with cholesterolized beef heart extract than with cholesterol-free extracts. Staphylococcus aureus, Escherichia coli and Bacillus alcaligenes faecalis caused negatively reacting serums to become anticomplementary. Bacilllus terminosporus had no such effect, while the growth of all the aforementioned bacteria produced anticomplementary properties in dextrose broth. The same culture medium behaved at first like a serum with a positive Wassermann reaction after inoculation with B. cereus, but after further incubation it acquired anticomplementary qualities. There was no noticeable relation between the serologic properties of the culture medium and the changes in the hydrogen ion concentration. I. DAVIDSOHN.

Tumors

TUMOR INDUCTION AND TUMOR GROWTH IN HYPOPHYSECTOMIZED MICE. R. KORTEWEG and F. THOMAS, Am. J. Cancer **37**:36, 1939.

In this experimental study the influence of the hypophysis on the induction of tumors and on the growth of tumors was studied in mice. When the genetic constitution of the mice was compatible, transplantation was successful in every case. The tumor grafts grew more slowly in hypophysectomized animals than in controls of the same age, but the relation of the final tumor weight to the body weight was the same in the animals operated on and controls of equal age. Papillomas and carcinomas induced by 3,4-benzpyrene appeared markedly later in the hypophysectomized mice than in the controls. With regard to the growth of tumor transplants and the response to carcinogenic agents, no qualitative difference was found between hypophysectomized mice and controls. The observed differences were entirely of a quantitative nature. FROM AUTHORS' SUMMARY.

LYMPHOBLASTOMA IN MICE FOLLOWING ADMINISTRATION OF CARCINOGENIC TAR. A. M. BRUES and B. B. MARBLE, Am. J. Cancer **37**:45, 1939.

A strain of mice derived from Bagg albino stock shows occasional lymphomatosis with a subleukemic blood picture, which runs a chronic course and appears not to be transplantable into normal mice of the same or other strains. The incidence of this condition is normally 2 per cent. Following cutaneous application of carcinogenic tar, the incidence of lymphoblastoma and lymphatic leukemia in these mice became as high as 50 per cent, and the disease followed a much more rapid course. The incidence of lymphoblastoma was closely correlated with the carcinogenic potency of the three different tars used. A series of C57 mice treated in the same way failed to show any such lesions. It is concluded that in the presence of a latent predisposition to lymphoblastoma a carcinogenic agent may act as an extrinsic factor leading to the development of this tumor.

FROM AUTHORS' SUMMARY.

EVALUATION OF THE RISK OF BIOPSY IN SQUAMOUS CARCINOMA. R. PATERSON and J. R. NUTTALL, Am. J. Cancer **37**:64, 1939.

A controlled clinical experiment shows that the incidence of metastases from squamous carcinoma is not increased by biopsy, but no generalization about other tumors can be made from this experiment. FROM AUTHORS' SUMMARY.

EFFECTS OF 3,4-BENZPYRENE ON HUMAN SKIN. G. B. COTTINI and G. B. MAZZONE, Am. J. Cancer **37**:186, 1939.

The assumption appears warranted that benzpyrene if applied to human skin for protracted periods would be carcinogenic as it is in animals.

FROM AUTHORS' CONCLUSIONS.

ACANTHOSIS NIGRICANS AND CANCER OF THE LIVER IN A DOG. H. O. CURTH and C. A. SLANETZ, Am. J. Cancer **37**:216, 1939.

Acanthosis nigricans and carcinoma of the liver in a dog is described. Acanthosis nigricans in dogs and human beings is essentially the same process. The observation recorded is another instance of the association of acanthosis nigricans and glandular cancer, and is at least consonant with the hypothesis that there is a common genetic factor for acanthosis nigricans and the cancer.

FROM AUTHORS' CONCLUSIONS.

SUSCEPTIBILITY TO TRANSMITTED LEUKEMIA OCCURRING IN MICE. M. D. SCHWEITZER and J. FURTH, Am. J. Cancer **37**:224, 1939.

Spontaneous leukemias originating in the highly leukemic stock Ak, in stock Rf, in which the incidence of leukemia is low, and in first generation and other hybrids were inoculated into mice of each of the pure stocks and of various hybrid combinations. The leukemias arising in different hybrids behaved in transmission experiments like the Ak leukemias. All of these leukemias can be transmitted to almost every member of the leukemic stock Ak and to F_1 generation hybrids, but not to members of stock Rf. All hybrid combinations tested have a substantial proportion of susceptible individuals, indicating dominance of inheritance; but further investigation is required to determine if one or two dominant genes are responsible for susceptibility. The duration of illness and the anatomic characteristics of leukemia are not modified by the genotype of the host. The susceptibility factors of both Ak and Rf leukemias are not allelomorphic. Evidence is presented that these genetic factors are specific for susceptibility to transmissible leukemia and differ from those that, according to Loeb, determine susceptibility for normal tissue grafts.

FROM AUTHORS' SUMMARY.

ABORTIFACIENT ACTION OF SERUM AND URINE FROM CANCEROUS PATIENTS. K. W. THOMPSON, T. HALE JR. and B. B. WHITCOMB, Am. J. Cancer **37**:233, 1939.

The experiments of Elsasser and Wallace dealing with an abortifacient agent in the serum and urine of patients with cancer have been repeated in part. The urine or serum of the majority of the tested cancerous subjects contained a principle which caused termination of early pregnancy in rabbits. The principle was not specific for cancer alone, since 3 of 6 apparently noncancerous patients had this agent in the blood or urine, and 1 cancerous subject did not have it in either serum or urine. The principal lesions of the animals given injections were apparent in the uteri, where there was degeneration of the fetal structures, including the syncytial and Langhans cells, thrombosis of, and hemorrhage from, the placental vascular structure and, later in the process, an infiltration of the necrotic tissue with leukocytes.

FROM AUTHORS' SUMMARY.

SEROLOGIC SPECIFICITY OF EXPERIMENTAL TUMORS. L. DMOCHOWSKI, Am. J. Cancer **37**:252, 1939.

The serologic properties of tumors produced in Wistar rats by subcutaneous injections of 0.2 per cent benzpyrene in lard were investigated. By intravenous inoculations of rabbits and Wistar rats immune serums were obtained which gave positive complement fixation with heated saline extracts of tumors (benzpyrene tumor, Jensen sarcoma, Walker carcinoma) and, though to a lower degree, with

heated saline extracts of certain normal rat tissues and organs, such as embryo, lung, stomach and intestine. Immune serum against normal rat muscles contains antibodies which fix complement with heated rat tumor and rat embryo extracts even more strongly than with homologous antigen. These cross reactions might be explained by the presence of normal antigens in tumor tissue. Differences in heated saline extracts of normal and tumor rat tissue are discussed in relation to the serologic properties of a dying tissue. FROM AUTHOR'S SUMMARY.

HISTOLOGIC CHANGES AND TRANSPLANTATION OF TISSUE SURROUNDING METHYLCHOLANTHRENE PELLETS. H. L. STEWART, Am. J. Path. **15**:707, 1939.

Five per cent methylcholanthrene-cholesterol pellets were placed subcutaneously in C_3H mice. The purpose of the experiment was to compare the results of histologic examination and of transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development. Tumor-yielding transplants were obtained from pellet mice sacrificed at forty-two and forty-nine days and following a lapse of four weeks at seventy-seven, eighty-two, ninety-one and ninety-eight days. In the sections of the pellet tissue which gave rise to growing tumors when transplanted, atypical cells with characteristics resembling those of malignant cells were present in varying number in the different mice. The results suggest that other factors in addition to the malignant-appearing cells about the hydrocarbon are needed for the development of a tumor on transplantation of pellet tissue into a new host. The results of the transplantation studies indicate that malignant changes may have been induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histologic recognition of malignancy became fully established.

FROM AUTHOR'S SUMMARY.

PINEALOMA OF DIFFUSE EPENDYMAL ORIGIN. R. P. MACKAY, Arch. Neurol. & Psychiat. **42**:892, 1939.

Mackay describes a unique tumor of the ependymal lining of all the cerebral ventricles, with obstruction of the sylvian aqueduct and involvement of the tuber cinereum and of the adjacent cerebral tissue, for several millimeters in some locations. The pineal body was not involved but was enveloped by the tumor mass. The tumor was found in a white boy aged 18 who had suffered for three years from confusion, amnesia and delirium and on admission presented the clinical picture of tuberculous meningitis. The tumor consisted of minute round lymphocyte-like cells, which packed the meshes of the reticulin network and were especially numerous around the blood vessels. Other cells were larger; some contained several vesicular nuclei; mitotic figures were common. In addition, ependymal cells (immature and adult) were scattered throughout the tumor, with a tendency to form perivascular palisades. The author holds that the pineal tumor arose from the ependymal lining, though the pineal body itself was not involved. Such a conclusion, in the author's opinion, is permissible in view of the fact that embryologically the pineal body is a differentiated ependymal structure.

GEORGE B. HASSIN.

VIRUS OF INFECTIOUS MYXOMA. R. E. HOFFSTADT and K. S. PILCHER, J. Infect. Dis. **64**:208, 1939.

The optimal temperatures for the growth of the virus of the infectious myxoma of rabbits on the chorioallantoic membrane is 33-35 C. After repeated serial passage the virus becomes adapted to the embryo as a medium for growth. After it has become thus adapted its titer is not affected by chilling of the embryo. Virus grown on the chorioallantoic membrane produces an infection in the embryo as indicated by gross changes and the blood count and by the fact that the vitality of embryos inoculated with the chick-passed virus is greatly reduced, as indicated by the reduction of the percentage of hatch. As a preservative of the virus of

infectious myxoma, normal rabbit serum is definitely superior to 50 per cent glycerol and physiologic solution of sodium chloride.

From Authors' Conclusions.

The Adenomatous Gastric Lesion in Strain I Mice. H. B. Andervont, Pub. Health Rep. **54**:1851, 1939.

The adenomatous lesion of the stomach which occurs spontaneously in practically all adult mice of strain I appears earlier and is more pronounced in the males. Susceptibility to the development of this lesion is inherited as a recessive characteristic, and a number of factors are involved. From Author's Conclusions.

Melanoma. E. K. Dawson, J. R. M. Innes and W. F. Harvey, Edinburgh M. J. **46**:695, 1939.

The benign melanoma or nevus cell tumor of the skin is a complex malformation, the principal components of which are melanoblasts or melanocytes; these are pigment-forming cells, and in man, in whom melanogenic potency has become restricted ("determined") to epithelium, the common nevus cell tumor is a manifestation of malinduction of the epidermis and its appendages. One form of the nevus cell tumor, the acanthotic nevus, is wholly epidermal. In the human eye and meninges there are found choroidal and meningeal melanotic cell aggregations of the mesodermic pigment cell type. Malignant melanoma in the human being is a carcinoma—epidermal melanocarcinoma in the skin, neuroectodermal carcinoma in the eye. Gaps still exist in knowledge of mesodermal melanoma, and a strict unitary conception of the melanoma may have to be abandoned. The existence of neural melanoma may not be summarily dismissed.

From Authors' Summary.

Pernicious Anemia and Gastric Carcinoma. A. W. F. Jenner, Acta med. Scandinav. **102**:529, 1939.

The conclusion is reached that the relative frequency of gastric cancer in association with pernicious anemia is due to the chronic gastritis which is nearly always present with pernicious anemia; also that patients with pernicious anemia should be observed closely for the possible development of gastric cancer. Cancer other than gastric does not have a relatively high incidence in patients with pernicious anemia.

From Author's Summary.

Technical

Iron Hematoxylins. R. D. Lillie and W. R. Earle, Am. J. Path. **15**:765, 1939.

Lillie and Earle show that iron-alum-hematoxylin solutions containing 13 to 17.3 Gm. of ferric ammonium sulfate (1.5 to 2 Gm. of ferric iron) in a 200 cc. quantity stain intensely in five minutes and do not overstain appreciably in thirty minutes. The addition of 15 to 20 Gm. of ferrous sulfate (3 to 4 Gm. of ferrous iron) preserves the original blue-violet color of the fresh solution for at least three months and preserves satisfactory staining for at least eleven and one-half months. Ferrous ammonium sulfate and ferrous sulfate may be used as sources of ferrous iron. Ferrous sulfate is to be preferred, as it gives a higher concentration of iron for the same weight of salt and is more soluble. It may be generally observed that solutions retaining their blue-violet color give satisfactory staining. Those showing a purplish violet to purplish brown color may also be quite usable, but those showing a yellowish brown color in thin layers are generally inert. Solutions containing 1.5 to 2 Gm. of ferric iron in the 200 cc. unit quantity to 1 Gm. of hematoxylin give adequate nuclear staining in five to seven minutes and do not stain other tissue elements appreciably in thirty minutes. Solutions containing around 0.5 Gm. of ferric iron (4.3 Gm. of ferric ammonium sulfate) stain

promptly and intensely in four to five minutes and overstain in longer periods. For preservation of the blue iron-hematoxylin lake about twice as much of ferrous as of ferric iron is necessary, i. e., quantities of ferrous sulfate or ferrous ammonium sulfate to give 3 to 4 Gm. of ferrous iron (15 to 20 Gm. of ferrous sulfate). A satisfactory solution which can be kept unchanged for several months is the following:

(A)	Ferric ammonium sulfate (violet crystals)	15 Gm.
	Ferrous sulfate	15 Gm.
	Distilled water	100 cc.
(B)	Hematoxylin	1 Gm.
	Alcohol, 95%	50 cc.
	Glycerin, chemically pure	50 cc.

Mix A and B in equal quantities.

While the foregoing type of solution is remarkably stable and effective, it is felt that greater stability may be attained, and studies now in progress indicate that such solutions can be attained. FROM AUTHORS' SUMMARY.

STOMACH LAVAGE IN THE DIAGNOSIS AND CONTROL OF TREATMENT OF TUBERCULOSIS. A. STADNICHENKO, S. J. COHEN and H. C. SWEANY, J. A. M. A. **114**:634, 1940.

Gastric lavage will find tubercle bacilli that become free in the larger bronchi in a manner not equaled by any other method used at present. Patients with tuberculosis in whom lavage of the stomach does not disclose tubercle bacilli include those with early minimal or moderately advanced lesions which have remained isolated or have been confined to the pulmonary parenchyma or otherwise have not disseminated bacilli into the larger bronchi; those with completely encapsulated lesions, and those with extrapulmonary tuberculosis—types of disease none of which discharge bacilli into the bronchi or pharynx. Completely healed tuberculous lesions and nontuberculous diseases yield uniformly negative results by gastric lavage. As gastric lavage seems to come nearer than any other method to finding all virulent tubercle bacilli, a negative result should be adopted as the ultimate standard for absolute negativity or apparent cure of patients who have had or who have been suspected of having tuberculosis. It is also useful in the elimination of frauds. While gastric lavage is not practical or even necessary for universal use, the efficiency of any method used or recommended for practical use may well be standardized by it. It is an excellent method for controlling the efficiency of collapse therapy. It should be emphasized again that during collapse therapy the absence of tubercle bacilli in only one washing of the stomach does not necessarily signify that the disease is arrested. The washings must be repeated at frequent intervals. Especially is this true when the collapsed lung is reexpanding. No patient with clinical signs of tuberculosis whose sputum is free from bacilli should be considered free from the disease until gastric aspiration has yielded negative results. FROM AUTHORS' SUMMARY.

ASPIRATION BIOPSY OF THE LIVER. P. IVERSON and K. ROHOLM, Acta med. Scandinav. **102**:1, 1939.

A method is described for aspiration biopsy of the liver. By means of a simple instrument it is practicable to aspirate a tissue column measuring about 2 by 15 mm., sufficient for an ordinary histologic examination. This form of biopsy has now been performed one hundred and sixty times by the authors; it proved unsuccessful in 22.5 per cent of the cases. It causes little inconvenience to the patient, and the risk involved appears to be slight. A tendency toward bleeding is a contraindication. It is possible by this method to demonstrate the presence of acute and chronic inflammatory conditions, tumors, degenerative processes, obstruction to the flow of bile, and other conditions. FROM AUTHORS' SUMMARY.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

CHARLES T. OLCOTT, *Secretary*

Regular Meeting, Dec. 28, 1939

Congenital Anomalies of the Coronary Arteries with Cardiac Hypertrophy in Infants. BERYL H. PAIGE.

Two cases are reported in which an anomalous origin of the left coronary artery from a pulmonary artery resulted in necrosis, fibrosis and calcification of the left ventricle, hypertrophy of the heart and cardiac failure.

The first patient was a 7 week old Negro girl, with a history of dyspnea. This was first noticed at the age of 2 weeks; thereafter a hacking cough developed. Three days before death the dyspnea became severe and progressively worse, the infant perspired excessively, showed pallor of the mucous membranes and nail beds, and felt cold. The temperature was 98.6 F. Enlargement of the heart was demonstrated by physical, fluoroscopic and roentgen examination. Death occurred on the fourth day after the onset of acute symptoms.

The heart weighed 64 Gm., which is about three times the average normal weight for the age. The right auricle was dilated, and the wall of the right ventricle was hypertrophied, measuring 6 mm. in thickness. No gross changes were visible in the myocardium. The left ventricle was dilated; the columnae carneae appeared as a meshwork of branching strands beneath thickened endocardium, and the papillary muscles were atrophied and gray or yellow. The wall of the left ventricle measured from 6 to 8 mm. in thickness and showed extensive scars. The right coronary artery originated from its normal site in the aorta and passed to the upper end of the posterior longitudinal sulcus. The left and larger coronary artery arose from the pulmonary artery, behind the posterior pulmonic cusp, and gave rise to the anterior descending, circumflex and posterior descending branches. Microscopically, the myocardium of the left ventricle showed hypertrophy, vacuolation, necrosis and atrophy of the myocardial fibers, as well as fibrosis and calcification.

The second infant, a white pseudohermaphrodite, was admitted to hospital because of a sudden onset of cough, listlessness, pallor and attacks of cyanosis. The temperature was 97.8 F. The alae nasi were dilating, and respirations were rapid and irregular. The heart rate was rapid; a forceful cardiac impulse was felt over the entire chest, and a rhythm suggesting a gallop rhythm was detected. Enlargement of the heart was demonstrated by fluoroscopic and roentgen examination. An electrocardiogram showed right preponderance and inverted TI waves, and the deformity of the QRS complexes indicated myocardial damage. The patient improved temporarily in an oxygen tent, but, following a recurrence of dyspnea and cyanosis, died at the age of 2½ months, twelve days after the onset of symptoms.

The heart weighed 64 Gm. The right auricle was dilated, and the wall of the right ventricle was hypertrophied. The left ventricle was dilated, and its endocardial surface was trabeculated; the papillary muscles were atrophied and contained yellow flecks; the myocardium showed extensive scarring. The right coronary artery was normal in its origin but divided into two branches, one of which passed to the left, anterior to the base of the aorta and pulmonary artery, to the

upper end of the anterior longitudinal sulcus. The other branch was small and coursed through the right auriculoventricular groove to the superior end of the posterior longitudinal sulcus. The left coronary artery arose from the right pulmonary artery, just distal to the bifurcation of the main pulmonary trunk. It gave rise to the anterior descending branch and the circumflex branch, which could be traced down the posterior longitudinal sulcus for a distance of 1 cm. Microscopic lesions essentially similar to those found in the first case were present in the myocardium of the left ventricle.

The myocardial injury in such cases is believed to be due to anoxemia of the portions of the myocardium receiving venous blood. Hence the extent of the lesions is dependent on the extent of distribution of the left coronary artery. Consequently, there may be a localized aneurysm of the left ventricle or the diffuse damage of the myocardium demonstrated in the 2 cases presented. Relatively few cases of congenital heart disease have been reported in which an anomalous origin of the left coronary artery from a pulmonary artery is the significant and usually the only cardiac malformation. The condition is not limited to children; in 14 of 21 collected cases the patients were infants, all of whom died during the first year of life.

DISCUSSION

ALFRED PLAUT: Was there diffuse fibrosis of the endocardium?

BERYL H. PAIGE: There was fibrosis of the endocardium, with marked thickening.

ALFRED PLAUT: Did the thickened endocardium contain many elastic lamellas?

BERYL H. PAIGE: A stain for elastic lamellas was not used.

ALFRED PLAUT: I am asking this question because there is a group of cases in the large chapter of the so-called idiopathic cardiac hypertrophy of the infant in which diffuse fibrosis of the endocardium seems to be a prominent factor. Among the cases I have seen I did not observe any such malformations of the coronary arteries as were described by you.

BERYL H. PAIGE: I have seen such malformations described in cases in which the endocardium was thickened. Malformations are not limited to such cases as these.

ALFRED PLAUT: May I ask another question, without really expecting an answer? What is your opinon as to the cause of the cardiac hypertrophy?

BERYL H. PAIGE: It has been looked on as diffuse hypertrophy following damage to the left ventricle. Bland and White consider it compensatory hypertrophy of the entire heart.

ALFRED PLAUT: The same condition is found without any anomaly of the coronary arteries.

DR. MUELLER: I should like to ask whether there was any particular pathologic feature in the mothers. Sometimes these pathologic conditions in infants are reflections of maternal conditions.

BERYL H. PAIGE: I was not able to find any evidence of disease or abnormality in the mothers. I went over the histories carefully, and there was nothing to indicate maternal disease or congenital malformations.

Acute Hemolytic Anemia and Tubular Nephrosis with Uremia Resulting from the Administration of Sulfapyridine (2-[Paraaminobenzene-Sulfonamido]-Pyridine). J. M. RAVID.

A case is reported of fatal acute hemolytic anemia and uremia resulting from administration of sulfapyridine in the treatment of pneumonia in a man 70 years of age. It occurred on the third day of treatment and after a total intake of only 8 Gm. of the drug. The clinical features were those of acute hemolytic anemia, with a sudden drop in hemoglobin to 58 per cent and in the erythrocyte count to 2,700,000, leukocytosis, reticulocytosis, fever, jaundice, oliguria progressing to total anuria, and uremia. The urine contained urobilin, and 10 per cent

of its volume consisted of packed red blood cells and hemoglobin. The blood urea nitrogen was 222 mg., nonprotein nitrogen 258 mg., uric acid 10.8 mg., cholesterol 310 mg., dextrose 421 mg., and creatinine 8.8 mg., per hundred cubic centimeters. The icteric index was 30.5, and a direct positive van den Bergh reaction was obtained. The sulfapyridine content of the blood was 11.5 per cent. The patient had complete anuria for the last thirty-six hours of his life. The main anatomic lesions were twofold: first, those secondary to the resulting hemoglobinemia and hemoglobinuria, viz., obstruction of the renal tubules by casts of hemoglobin pigment and its derivatives and hemosiderosis of the liver, kidneys and reticuloendothelial system, and, second, those produced apparently by direct toxic action on the hepatic and renal epithelium. The latter lesion was slight. The lesion in the liver consisted mainly in cytoplasmic vacuolation and accumulation of fat, which was more or less central in distribution. The salient lesion in this case, the renal lesion, which resulted in complete anuria and fatal uremia, consisted in blockage of the tubules with pigment from hemoglobin and its derivatives and, to a lesser degree, in degenerative changes in the tubular epithelium proper.

This blockage was noted mainly in the region of Henle's loops, the recurring limbs and the collecting tubules; the proximal segments of the tubules were dilated. A few calcium deposits in the tubular casts were also noted.

The clinicopathologic picture induced by sulfapyridine is not a syndrome sui generis for this drug alone but appears analogous to those encountered in death following transfusion of incompatible blood (a number of cases), in death following experimental transfusion, in blackwater fever, paroxysmal hemoglobinuria, occasionally in quinine poisoning and in poisoning with certain other chemicals.

The formation of urinary calculi composed of acetyl sulfapyridine crystals, alone or in combination with calcium salts, must also be taken into account in the consideration of the toxic effects of sulfapyridine on the urinary apparatus in general.

Careful daily observations of a patient receiving sulfapyridine with regard to the blood picture and the renal function and the immediate discontinuance of this drug on detection of any deleterious effects on the kidneys or blood should, among other things, be the guiding principle in treatment with sulfapyridine.

DISCUSSION

MILTON HELPERN: Some months ago I had occasion to make an autopsy on the body of a young man who had ingested two tubes of a well advertised brand of tooth paste, which has a high content of potassium chlorate. The clinical course was attended by methemoglobinemia, hemoglobinuria and retention of nitrogen in the blood. The kidneys were very similar to those which Dr. Ravid described tonight; they were swollen, especially the cortices, and severe degeneration of the convoluted tubules was evident microscopically. The collecting tubules were all distended with hemoglobin casts. The urine found in the bladder at autopsy contained a large number of thick, long, coarsely granular hemoglobin casts.

J. M. RAVID: Dr. Helpern's case is a striking one, and the condition appears to belong to the same class of nephropathies which I have described, namely, chemical obstructive and degenerative nephrosis.

Visceral and Vascular Lesions in Scleroderma. ABOU D. POLLACK.

The association of visceral and vascular lesions with scleroderma suggests that scleroderma may in certain instances represent merely a local manifestation of a disease state in which widespread alterations can be found in many other organs and tissues of the body.

Two cases of scleroderma showing certain similarities in clinical course are presented. Preceding the full development of the sclerodermatous change, each patient experienced premonitory circulatory disturbances in the extremities. Both had arthropathy, evidence of active renal disease, anemia, leukocytosis and fever.

Anatomically, the most striking change is a fibrinoid deposit in the walls of small arteries and arterioles in many organs. In the first case the fibrinoid deposits were limited almost exclusively to the intima. In the second, the fibrinoid degeneration was found throughout the vessel wall. The intimal proliferation and periarterial inflammation recall the vascular lesions seen in periarteritis nodosa. The vascular lesion is most evident in the kidneys. In the second case the kidneys showed, in addition to the vascular changes, a peculiar type of glomerulonephritis, with the so-called "wire loop" changes, as seen in disseminated lupus erythematosus.

The genesis of the vascular and mesenchymal alterations in both cases is, at the present time, unclear. It is apparent, however, that certain forms of scleroderma must be considered together with that puzzling group of conditions which have been called "toxic" or "allergic" diseases. These include disseminated lupus erythematosus, dermatomyositis and periarteritis nodosa.

DISCUSSION

GEORGE BAEHR: Clinically, scleroderma represents a degenerative and atrophic lesion in the skin; it is a symptom of disease and not a disease entity. It occurs most commonly in persons who have a sympathetic vasomotor disturbance, so-called Raynaud's disease. As a result of long-standing Raynaud's disease sclerodermatous changes occur in the skin of the extremities, face and other parts of the body. Similar localized disturbances occur in the skin of the extremities secondary to various forms of joint disease of the hands and feet.

The case of diffuse scleroderma with visceral and vascular lesions which Masugi described seems to be identical with the cases which Dr. Pollack has presented and with the case which Talbot, of Boston, described last year. It seems to me that these 4 cases do constitute a disease entity. In making this statement, I should emphasize again that this is only one form of diffuse scleroderma. I think it would be wrong to give the impression that all cases of diffuse scleroderma necessarily belong in this group, for scleroderma in itself is merely a superficial manifestation possibly of a variety of different conditions. Similarly, an erythema on the bridge of the nose spreading in a butterfly fashion on the malar eminences of the face does not in itself warrant a diagnosis of lupus erythematosus in the absence of the complete clinical picture. An erythema of this type and in this location can be due to other causes.

In the 2 cases which Dr. Pollack described, and those of Masugi and Talbot, one has a common group of clinical and pathologic phenomena—in other words, a distinctive clinical picture and pathologic process. There are: a long, intermittently febrile course, progressive changes in the skin characteristic of scleroderma, urinary evidences of changes in the blood vessels of the internal organs and a tendency toward involvement of joints and serous membranes.

I had the opportunity to study both patients clinically, the man six months before scleroderma developed. The persistent presence of red blood cells in the urine indicated to me, even at the first observation of the patient, that there must be some vascular lesion in the viscera. The tendency to swelling of the hands and feet and the peculiar glovelike cyanosis of the hands I felt obliged to ascribe to vascular phenomena resulting in stasis in capillary circulation, perhaps most significant on the venous side of the capillary bed. Only after many months did this obscure vascular disturbance result in almost universal scleroderma. In addition to the clinical evidence of vascular disease in the kidneys, these patients had involvement of the synovial membranes of the joints and of the serous membranes of the pericardium, pleura and peritoneum. The first patient had conspicuous leukopenia at one time and a tendency toward depression in the hemoglobin and red cell count. In other words, the clinical picture, as well as the pathologic process in the vessels and serous membranes, bears a close resemblance to the clinical phenomena and the lesions seen in lupus erythematosus, and yet the cutaneous manifestations are so different.

Concerning the cause of the condition, I think it is best to confess complete ignorance. It seems to me to be unwise to label the condition allergy or to use any other obscure term, for this would merely serve to hide the fact that one has not the ghost of an idea what the essential nature of the process may be. It is important now in considering all cases of diffuse scleroderma that one be aware of the fact that in some of them the condition may represent a diffuse systemic disease affecting not only the skin but vascular structures throughout the body, as well as the serous membranes and the synovial membranes, and that it may fit into a disease entity having some relationship perhaps to diseases like lupus erythematosus and dermatomyositis.

J. M. RAVID: I should like to ask Dr. Pollack concerning the course of the blood pressure in the first case and the course of the renal function in both cases during the progress of the disease.

ABOU D. POLLACK: Both patients had normal blood pressure throughout their illnesses until the terminal event, when the blood pressure rose to a high level only just before death. In the first case no special studies of the blood were made in relation to renal function; in the second case there was terminal retention of nitrogen.

Certain Diseases Observed in North China. C. H. HU (by invitation).

This was a brief presentation, with over 80 photographic illustrations, of certain diseases observed in the Peiping Union Medical College, Peiping, China. The diseases included: follicular hyperkeratosis due to avitaminosis A; nutritional edema; typhoid fever; bacillary dysentery; leprosy; tuberculosis; syphilis; relapsing fever; typhus fever; kala-azar; amebiasis; schistosomiasis; cysticercosis; echinococcus infection; Laennec's cirrhosis; thromboangiitis obliterans, and various kinds of tumors.

ALFRED PLAUT, *President*

CHARLES T. OLCOTT, *Secretary*

Anniversary Meeting, Jan. 25, 1940

Thromboarteritis of the Pulmonary Artery with Chronic Obstruction in the Pulmonary Circulation. B. M. VANCE.

A white man, 52 years old fell downstairs, sustaining a laceration of the scalp in the right parietal region, after which he was unconscious for several minutes. Several hours later he became markedly dyspneic and cyanotic. He died after six days, with evident signs of right-sided cardiac failure.

The necropsy disclosed a muscular, slightly obese white man with a laceration of the scalp as noted, but no other sign of injury. The skull and brain were normal. The right ventricle and auricle of the heart were markedly distended and hypertrophic, while the left ventricle and auricle were small in comparison. The aorta, coronary arteries, cerebral arteries and femoral arteries and veins were normal aside from slight intimal sclerosis. The lungs were well aerated anteriorly, but posteriorly they showed a few depressed areas of atelectasis. The air passages were normal. The main stem of the pulmonary artery and its right and left branches were occluded with a fragile, grayish red thrombus. The larger branches of the intrapulmonary arteries were filled with a firm, layered thrombus, while the arteries 2 to 6 mm. in diameter had thick fibrous walls and contained recent thrombi here and there. The pulmonary veins were normal. Other conditions found at necropsy were passive congestion of the liver and spleen, slight subcutaneous edema around the ankles, moderate arteriosclerotic changes in the kidneys, cholelithiasis, slight prostatic hypertrophy and an old false joint in the shaft of the left ulna.

Microscopic examination of the pulmonary arteries disclosed that the vessels of 2 to 6 mm. caliber were narrowed or occluded by marked proliferation of the subendothelial connective tissue, which was extensively canalized by minute distended blood vessels. In some arteries the canalized tissue filled the lumen, but in others it narrowed the lumen and pushed it to an eccentric position. The elastic layer of these arteries showed widespread splitting. The larger vessels contained organizing thrombi, with polymorphonuclear leukocytes grouped in the vessel wall around the thrombotic area. In some regions the large trunks of the pulmonary artery were edematous because of fluid in the tissue spaces. In other areas the vasa vasorum were distended and surrounded by numerous lymphocytes. The smallest pulmonary arteries were normal except for a few vessels which showed cellular proliferation of the subendothelial tissue and narrowing of the lumen. There was no atheromatous degeneration or calcification in any of the pulmonary vessels.

The principal lesion in this case was nonspecific arteritis of the pulmonary artery associated with thrombus formation, which caused gradual narrowing of the lumen of the artery and chronic obstruction in the pulmonary circulation. The etiologic factor in this case could not be determined, but the process had apparently started in the medium-sized arteries of the lungs and from there extended to the larger trunks.

DISCUSSION

ANDREA SACCONE: I should like to ask if the vessels of the other organs, the spleen and the kidney, were investigated, because many times this type of pathologic picture in the lung is associated with proliferative endarteritis in the other organs.

ALFRED PLAUT: What was the profession of the patient?

B. M. VANCE: He was a janitor. What his profession was before that I was not able to ascertain.

ALFRED PLAUT: I had a special reason for asking that question. In going over the literature of pulmonary thromboarteritis I found some relative predominance of the condition in gardeners and in people who might have been exposed to lead.

In 1935 at the Medical Fortnight I exhibited a case of pulmonary endarteritis which differed from the case which Dr. Vance presented in that the disease attacked the smaller vessels.

B. M. VANCE: The other arteries of the body showed a slight amount of endarterial change, a thickening of the intima of the usual arteriosclerotic type, which was not particularly pronounced. There was slight phlebosclerosis of the right femoral vein. The bronchial artery on section exhibited definite narrowing of its lumen due to arteritis obliterans. The aorta was normal except for slight intimal sclerosis. The arteries in the other organs of the body were normal.

Contralateral Adrenal Atrophy Associated with Cortical Adrenal Neoplasms. TOBIAS WEINBERG.

The purpose of the presentation of the 3 cases of adrenal cortical neoplasm is to reemphasize the frequent occurrence of contralateral adrenal atrophy in association with such a neoplasm. All 3 patients were women, and all presented the Cushing syndrome. Two were operated on and found to have cortical adenoma. Both died postoperatively. The third was found to have carcinoma of the adrenal cortex with metastases in the liver and lungs. In all 3 cases the contralateral adrenal was definitely atrophic. A review of the literature for the past fourteen years yielded 34 cases of adrenal cortical neoplasm in which the clinical diagnosis of the state of the adrenals was confirmed by autopsy. In 21 of these cases, or approximately 62 per cent of the total number, the contralateral adrenal showed atrophy.

It is suggested that in order to prevent the high mortality incident to operative intervention, desoxycorticosterone pellets, for example, be implanted at an adequate time before operation so that they may have effect; also that once the

diagnosis of an adrenal cortical neoplasm is made, operation should not be delayed, so that functional inactivation, if not actual atrophy of the contralateral adrenal, may be avoided.

Influence of Sulfanilamide and Sulfapyridine on the Evolution of Experimentally Induced Pneumococcic Pneumonia in Rats. DAVID GOLDSTEIN (by invitation) and IRVING GRAEF.

This paper will be published in a later issue of the ARCHIVES.

DISCUSSION

REUBEN OTTENBERG: Were pneumococci found in the abscesses in the treated animals, or were they sterile abscesses?

S. C. BUKANTZ (by invitation): From the charts it appeared that the first negative cultures from the lungs of treated animals were encountered on the fifteenth to eighteenth days. Later on it was brought out that bacteria were not present in the lungs of the treated animals twenty-four hours after treatment. Is there a difference between spread and culture? I wondered whether any studies of the concentration of the drugs in the blood were done to give some idea of what levels were reached in the respective groups of treated animals, and whether the treatment was ever varied so as to begin at intervals longer than two hours after inoculation of the pneumococci.

DAVID GOLDSTEIN: In reply to Dr. Ottenberg's question, there were several abscesses in fifteen day animals which failed to reveal pneumococci on careful study both by stains and culture. However, some of the abscesses in the treated animals put to death at four and five days revealed the presence of pneumococci.

A question was raised about confusion in the reports of the negative cultures. I think that can be clarified by considering again the mode of presentation of the results in the charts. Only positive cultures were recorded against the total number of animals for each day. Thus, at one day there were 12 sulfanilamide-treated animals, 5 of which were shown to have positive lung cultures. As time went on, the incidence of positive lung cultures grew less frequent. On the second day there were 3 positive cultures for 8 animals, on the fourth day 2 for 9 animals, and for the 8 animals of the fifteenth day there were no positive cultures. Sulfapyridine yielded similar results.

S. C. BUKANTZ: Then that is the first time when all animals in a group gave negative lung cultures?

DAVID GOLDSTEIN: Yes. The fifteenth day was the first time that all cultures were sterile. In reply to your other question, we determined the concentration of the drug in the blood in our experiments with sulfapyridine. We made no determinations in the experiments with sulfanilamide but used Marshall's data on rats as a standard, from which it was possible to estimate the level of sulfanilamide to be 15 mg. per hundred cubic centimeters. The level of sulfapyridine in 4 rats was determined to be approximately 20 mg. per hundred cubic centimeters. It varied between 14 and 26 mg. per hundred cubic centimeters.

S. C. BUKANTZ: What length of time elapsed between the administration of sulfapyridine and the determination of the concentration of the drug in the blood?

DAVID GOLDSTEIN: One estimation was made four hours after the administration of the drug, and that was 14 mg. per hundred cubic centimeters. With the same dose of 200 mg. daily there apparently was a cumulative effect, so that the concentration rose to 26 mg. per hundred cubic centimeters on the third day.

We have a small series of 8 rats in which delayed treatment was studied. These were thought too few for presentation. Half of this group survived, and half died. They were all critically sick, and those which died, died shortly after the first treatment. The results in those which survived and were put to death on the tenth day roughly paralleled the results in the treated rats presented this evening.

Aplastic Anemia. CORNELIUS P. RHOADS (by invitation).

A clinical and pathologic study of anemias refractory to treatment with accepted hemopoietic agents is reported.

The histologic changes of the bone marrow are described, and the changes present in the idiopathic disease are compared with those found in clinically similar conditions of known toxic nature.

The natural history and the symptom complex of the disease are discussed as observed in the 66 cases included in this report, and particular reference to remissions is made.

Studies of the metabolism of bile pigments in patients with refractory anemia are reported, and the results compared with the results in similar studies of normal persons.

The excretion of porphyrins in refractory anemia and in other disorders of the blood is discussed, since evidence recently presented by Dobriner shows that the rate of excretion of coproporphyrin type I may be used as an index of the rate of cell formation by the marrow and that type III is a pathologic product.

The results of tests of liver function in 21 of the cases in the series are presented and discussed.

The results of test of ability to conjugate cyclic compounds into sulfates and glucuronates are reported and the significance of these results considered.

The relationship of refractory anemia to leukemia is mentioned.

Finally, certain considerations of causes and of treatment are discussed, and the histologic changes in idiopathic refractory anemia compared with those of refractory anemia of known toxic cause in animals.

DISCUSSION

CHARLES L. FOX JR.: Dr. Rhoads mentioned Dr. Rimington's and Dr. Brownlee's work on porphyrins in the urine of animals treated with sulfanilamide and various antipyretic drugs. They showed that methemoglobinemia paralleled the excretion of the type III porphyrin. Do your studies show such a parallelism?

CORNELIUS P. RHOADS: No, my associates and I have never found consistent methemoglobinemia in these patients. There is a hitch to the work of Rimington and Brownlee, since their work was done principally on rats. They showed that rats had an increased output of type III porphyrin when poisoned by certain compounds with aromatic amines. We are not at all sure that normal rats do not excrete type III porphyrin instead of type I as does man. We have shown that type III porphyrin is excreted by normal rabbits, and work on rats is in progress.

CHICAGO PATHOLOGICAL SOCIETY

S. A. LEVINSON, *President*

EDWIN F. HIRSCH, *Secretary*

Regular Monthly Meeting, Jan. 8, 1940

Radiosensitivity of Primary Carcinoma of the Lung and the Effect of Irradiation on Time of Survival. PAUL E. STEINER.

Twenty-one primary carcinomas of the lung which had been treated with known amounts of roentgen rays were studied post mortem. Marked degenerative changes were observed in some, but in none was the therapy lethal for all of the tumor cells, although the therapeutic dose was as much as 5,000 roentgens. Contrary to the anticipated result, undifferentiated cell carcinoma appeared to be more radioresistant or to have a higher degree of recuperability following roentgen treatment than adenocarcinoma and squamous cell carcinoma. Marked

cell degeneration was seen in 2 squamous cell carcinomas treated with 1,670 r and 1,490 r, respectively, although in others larger doses produced no visible effects. The carcinomacidal dose for primary cancer of the lung appears to be over 5,000 r. In tissue from a small frontal bone metastasis of an undifferentiated cell type of pulmonary cancer 3,800 r had destroyed all tumor cells. The tissues of the lung, pleura, pericardium, heart and esophagus adjacent to the tumors had no recognized damage from the irradiation. The histologic effects of irradiation on the tumor cells resembled those described by others for other types of tumor. The average time of survival from the date of the first treatment in five patients whose cancers were given over 3,000 r was one hundred and thirty-one days. Life was not notably prolonged in the group whose cancers were irradiated when compared with a group of 53 whose cancers were not irradiated.

This paper will appear, with details of histologic changes, the technic of therapy and illustrations, in the *Archives of Internal Medicine.*

DISCUSSION

P. J. MELNICK: It is encouraging to note an increasing interest in the study of tissue changes induced by radiant energy. The histologic changes that I have observed in human and animal tumors are the same as those demonstrated, and my understanding of them is the same. I shall emphasize two features: The rate of delivery of radiant energy is significant, i. e., high intensity over a short period in contrast with low intensity over a longer time. Large doses in a short time cause necrosis; fractional doses cause retrogressive changes in the cells, due to cumulative effects. Also, certain tumor tissues show the full cumulative effect, but the small cells, according to Dr. Steiner's report, do not. The reason is not known.

H. C. SWEANY: Is there any evidence that small doses stimulate growth of the tumor? How were the biopsy tissues taken?

I. PILOT: Did roentgen films demonstrate shrinkage of the tumors during treatment?

S. R. ROSENTHAL: Certain bronchial tumors contain anaplastic tissues, occasionally tubules, and are termed adenoma.

S. A. LEVINSON: Was there a difference in the incidence of metastases as between irradiation and nonirradiation?

PAUL E. STEINER: Nothing was observed to suggest that small doses of radiant energy accentuated the growth of the tumors. Some biopsy tissues were obtained through the bronchoscope; other tissues for control were metastases not subjected to radiation. There were 2 "adenomas" in the material studied. They were comparatively benign but spread by continuous slow growth. Graham has spoken of them as mixed tumors. There was no difference in the development of metastases in the treated and untreated groups.

Effect of Colchicine on Human Tissues. W. O. BROWN.

Colchicine produces a diminution in the size of experimental tumors, as well as nuclear changes in the cells of almost all parenchymatous organs. The effects of colchicine are most prominent in cells undergoing mitosis, which may be arrested for a variable time. Bizarre nuclear configurations result, and not infrequently cell death. Three patients with inoperable malignant growths died in the course of colchicine treatment. The results of the autopsies indicate that the action of colchicine is not selective but that its effects are most marked on those cells having a high rate of metabolism and mitosis. There is nothing specific about the morphologic appearance of the cells arrested by colchicine. Identical changes are frequently found in routine autopsy material, without reference to the cause of death. Owing to the depressing effect on bone marrow, agranulocytosis and aplastic anemia occur frequently. An observation not recorded

previously is the production of peripheral neuritis with fatty degeneration of the myelin sheaths.

The complete report will be published elsewhere.

DISCUSSION

PAUL E. STEINER: Is there enough evidence in patients or experimentally in animals to justify continued use of the drug?

A. B. RAGINS: In my studies of tumor tissues with colchicine therapy, the growth of cells seemed to have been halted in the metaphase.

F. QUEEN: Were these patients killed by the colchicine therapy?

W. O. BROWN: There are only a few experimental studies on the effects of colchicine in animals. Dustin in 1908 noted that larger doses caused anaplastic anemia and that small doses stimulated the bone marrow. Apparently the arrest of tumor tissue growth is in the metaphase. The nuclear changes later lead to the conditions observed. In 2 patients death resulted from agranulocytosis caused by the colchicine, although both patients had extensive carcinoma.

Tetralogy of Eisenmenger. M. LEV.

The types of congenital abnormality of the heart that permit survival to adult life are relatively few. Among these are the tetralogy of Fallot and the tetralogy of Eisenmenger. The tetralogy of Fallot consists in a dextroposition of the aorta, a defect of the interventricular septum, right ventricular hypertrophy and stenosis of the pulmonary tract. The tetralogy of Eisenmenger differs from that of Fallot in not including pulmonary stenosis. Instead, the pulmonary artery is either normal in size or, more often, is dilated. A 19 year old youth was thought clinically to have the tetralogy of Fallot with mitral stenosis. At autopsy the tetralogy of Eisenmenger was found with stenosis of the mitral orifice. Both the aorta and the pulmonary artery emerged from the right ventricle. The only outlet of the left ventricle was a defect of the ventricular septum in the region of the pars membranacea. The pulmonary artery was wider than the aorta. The outlet regions of the arterial trunks were separated by an arch of musculature at the base of the right ventricle. In addition there were coarctation of the aorta, a patent ductus arteriosus, an abnormally formed left auricular appendage and marked mitral stenosis. From the standpoint of transposition, this anomaly is type II, according to Spitzer, or a partial transposition, according to Rokitansky. The embryologic explanation of this anomaly is part of the general explanation of transposition which will be discussed in another communication. The tetralogy of Eisenmenger may be classified according to the degree of transposition into: (*a*) transposition with an aneurysm of the membranous septum (the case of Libman and Abbott belongs here), (*b*) a riding aorta with a defect of the interventricular septum (this includes most of the cases recorded in the literature) and (*c*) partial transposition, in which the aorta and pulmonary artery arise from the right ventricle. This case apparently is the only one of the last variety to be recorded in the literature. It also seems to be the only one on record complicated by stenosis of the mitral orifice, the result of an old acquired endocarditis.

DISCUSSION

O. SAPHIR: The anomaly which Eisenmenger described was regarded at first as the tetralogy of Fallot. Appreciation of these anomalies is not so difficult if one remembers that the disturbance is due to torsion defects of the bulbus.

Acute Necrosis of the Adrenal Glands with Tryparsamide Therapy. M. G. BOHROD.

Wells, Humphreys and Work reported selective necrosis of the adrenal glands in a woman who received germanin (Bayer 205) for pemphigus. They suggested that the increase in Addison's disease due to cortical atrophy may be the result of toxic destruction by some drug or drugs as yet undisclosed. A man aged 51

years received twelve injections of tryparsamide for dementia paralytica. He died within a half hour after the twelfth injection, apparently in shock. Necropsy showed, in addition to the lesions of dementia paralytica and syphilitic aortitis, marked swelling and hemorrhagic necrosis of both adrenal glands. The two weighed 50 Gm. Histologically, both cortex and medulla were necrotic, but small islands of cortical and medullary cells were apparently intact. No other cause for the adrenal necrosis was found, and it seems that the tryparsamide injected may have been the cause. Attempts to produce adrenal necrosis in animals by injections of tryparsamide have failed. Germanin and tryparsamide have no obvious chemical similarity. Both drugs are effective in the treatment of trypanosomiasis. This suggests a pharmacologic similarity, because the only 2 cases in which a drug may have been the cause of necrosis of the adrenal glands had this similarity of therapeutic action. While this report strengthens the conception of Wells and his associates that drugs may be responsible for the increase of cortical atrophy of the adrenals, there is the possibility that acute infections, principally meningococcic infections, may be associated with necrosis of these glands. In a case in which the condition accompanied lobar pneumonia, thrombosis was present in one gland but not in the other, suggesting that thrombosis of the adrenal vein, often claimed as the cause of adrenal necrosis, may be secondary to the necrosis.

DISCUSSION

E. M. HUMPHREYS: This report adds another drug to the small number which seem to have some relation to selective necrosis of the cortex of the adrenal gland. Since our report (*J. A. M. A.* **109**:490, 1937) 2 more cases in which treatment with germanin (Bayer 205) was followed by necrosis of the adrenal cortex have been published (Tomlinson reported one of these [*Arch. Dermat. & Syph.* **38**:555, 1938]). Some experimental studies have demonstrated that germanin causes selective necrosis of the cortex without affecting the cells of the medulla.

PAUL R. CANNON: I have recently observed focal necrosis in the cortex of both adrenal glands with phenobarbital poisoning and subsequent agranulocytosis.

S. R. ROSENTHAL: Sulzberger observed varying results with arsphenamine in animals, results which he was unable to explain. Perhaps some dietary condition is a determining factor.

O. SAPHIR: There are two different diseases in the adrenal glands: (1) hemorrhage with necrosis of both cortex and medulla and thrombosis of the veins and (2) selective necrosis of the cortex without injury of the medulla. Atrophy of the cortex with focal regeneration probably does not occur after diffuse necrosis.

M. G. BOHROD: I do not think that a causal relation between the drug and the lesions has been established in this case. Nor does thrombosis explain all of the changes.

PATHOLOGICAL SOCIETY OF PHILADELPHIA

J. H. CLARK, *President*

H. L. RATCLIFFE, *Secretary*

Regular Meeting, Jan. 11, 1940

Larvae of Multiceps Serialis in a Gelada Baboon: Possible Relation to Hydatid Disease. H. L. RATCLIFFE.

Multiceps serialis is one of a group of cestodes which utilize various herbivorous mammals as intermediate hosts and complete their development in the intestines of dogs. Because the larvae attain considerable size, the larval stages of these tape-

worms are much better known than their maturity. Certainly the best known of these larvae is the hydatid cyst of Echinococcus granulosus.

Occasionally, instead of growing as a single cyst, Echinococcus assumes a multilocular form which lacks a limiting capsule and infiltrates surrounding tissues in a manner suggesttive of a malignant tumor. Both forms of cyst localize most frequently in internal organs but at times may involve skeletal muscles or subcutaneous tissues.

The larva of Multiceps serialis also develops as a multilocular cystic mass that infiltrates and destroys adjacent tissues, a feature well illustrated in the present case:

An adult male baboon, Theropithicus gelada, had been exhibited at the Philadelphia Zoological Garden for about five years. This animal, as is characteristic of the species, had a thick mass of long hair covering the neck and shoulders, so that a subcutaneous mass would not be seen until large enough to displace this hair, unless the animal was restrained for examination. The presence of the parasite was not suspected until the overlying skin had ulcerated. The animal died of circulatory collapse about twelve hours after he had been anesthetized for examination. The large multilocular cystic mass which had developed in the subcutaneous tissues and superficial muscles of the shoulder and arm corresponded equally well to descriptions of Echinococcus multilocularis and Multiceps serialis. However, examination of scolices from the cyst showed that it was an example of the latter organism. Differentiation of the two parasites, while entirely of academic interest, must depend on the morphologic appearance of scolices from the cysts, since it is not possible at present to distinguish them by serologic tests.

Neuropathologic Study of a Case of Tetanus. W. P. JENNINGS and R. P. CUSTER.

A young white woman was admitted to the surgical service of Dr. John C. Howell at the Presbyterian Hospital with tetanus, which had developed sixteen days after lodgment of a wooden splinter in the left knee. Tetanus antitoxin was given prophylactically, but the splinter was not located until autopsy; 84,000 units of serum was given therapeutically. A serum reaction occurred, and, following further administration of the serum, shock developed; this subsided, but the woman died soon thereafter, on the day following admission, in an exacerbation of the disease.

Postmortem examination disclosed the foreign body in the soft tissues of the left knee, localized by proliferating fibrous tissue. Tetanus bacilli were recovered from pus in the wound. There was marked degeneration of all of the organs, with extensive hemolysis. The brain was tightly applied in the cranial cavity, the tissue was softened, the spinal fluid was increased, and there was marked cerebellar coning. The meninges appeared normal. Cut surfaces were markedly engorged, and there was staining of the tissues with blood perivascularly. Spinal cord tissue, particularly the cervical and thoracic segments, appeared edematous and congested. The lesions of the nervous system were predominantly degenerative. There was degeneration of the third and fourth cerebral cortical layers, with accompanying gliosis and widespread vascular endothelial proliferation. The ganglion cells were undergoing chromatolysis. Tract demyelination, particularly in the region of the fourth ventricle, was noted. Cerebellar granular and molecular layers were degenerated, and the Purkinje cells were undergoing extensive chromatolysis. A section of the sciatic nerve underlying the site of infection showed extensive loss of fibers, demyelination and perineural proliferation. A corresponding section of the right sciatic nerve was edematous, and there was demyelination with sheath proliferation of lesser extent. The process involving the right sciatic nerve was interpreted as predominantly an acute degenerative process, in contrast to a more chronic process with reactive phenomena in the sciatic nerve on the involved side. Finally, all the tissues showed evidence of hemolysis.

The papers of Abel may be recalled in which he favored a peripheral and a central action of the tetanus toxin and expressed the belief that action on the

nervous system resulted through blood stream–lymphatic distribution rather than by direct transmission by nerve or by perineural lymphatics to the meninges. Experimentally, lethal doses injected intravenously were apparently fixed in all of the tissues in an irrecoverable form; any toxin in excess of the lethal dose could be recovered from the circulation.

We interpret our findings as support for the experimental evidence of Abel that the toxin is lymph borne and blood borne and that there is general toxemia, for not only were degenerative changes for the most part rather uniform and generalized throughout the central nervous system but all of the other tissues examined shared equally in the degenerative process. The second reason is that the first symptom apparently was general malaise, followed by dysphagia, and soon thereafter by generalized involvment; if the toxin had traveled along the nerve, the affected segment of the spinal cord should, supposedly, have given prominent localizing signs early. The assumption of nerve transmission cannot be justified on the basis of the sections. Since more proximal segments of the nerves were not examined, no knowledge is at hand as to whether they were similarly involved to the same relative extent. Examination of the spinal cord itself showed symmetric degeneration.

Pulmonary Pneumatocele (Bullous Emphysema, Giant Bullae of the Lung, Polycystic Lung): Report of a Case. ESMOND R. LONG.

A Negro approximately 40 years old entered the outpatient department of the Philadelphia General Hospital in acute respiratory distress, wheezing and gasping for breath. He died ten minutes after entrance. The available past history was meager. He had served a number of prison sentences for violation of the narcotic laws, and had once been examined in the outpatient department of another hospital. In each of these institutions he gave a history of asthma and repeated colds. No roentgen films were made during life, and no definite diagnosis was made.

Autopsy disclosed multiple pneumatocele of the lungs. Each upper lobe was composed largely of giant bullae, which compressed the functional portion of the lung below. Acute terminal dilatation of these bullae, dependent on a valvelike opening to each, was apparently the cause of death.

Multiple pneumatocele of the lung is not rare, but this occurrence of it seems to merit record because of the huge size of the air sacs, which reached diameters of 12 to 15 cm. in each upper lobe, and because of the acute termination. The causes in most cases are obscure, and the designations of the condition are confusing. In many instances it has been called congenital cystic disease of the lungs, but in most cases it is not congenital, and the air sacs are not cysts according to the usual definition of a cyst. The multiple pneumatocele under consideration appeared to be the sequel of old diffuse chronic infection and resultant fibrosis of the bronchiolar walls.

Genesis of Polycystic Disease of the Kidneys. ROBERT F. NORRIS and LEON HERMAN.

The important theories of the origin of polycystic disease of the kidneys are reviewed. Congenital polycystic kidneys are described as observed in 4 patients. From serial sections, several collecting ducts and cysts in the kidneys of each patient have been reconstructed to scale in drawings. Evidence is given that for a long period in fetal life the development of these kidneys was normal. After differentiation of the metanephrogenic anlage and after the union of its elements with collecting ducts, there occurred focal cystic dilatations of uriniferous tubules and collecting ducts followed by isolation of the dilatations as cysts of segments of these nephrons. Continued proliferation of these elements and rupture of their walls resulted in anastomoses among these cysts. The changes are thought to be degenerative since they resemble the stages in normal degeneration of the mesonephros and of the normal vestigial elements of the metanephros. In the case of

polycystic kidneys, therefore, to a variable degree the metanephros is abnormally provisional. Such an explanation appears to be applicable to most of the congenital anomalies associated with polycystic kidneys and to be compatible with the hereditary nature of the disease. The fundamental cause of polycystic disease of the kidneys must be in the germ plasm.

A Scientific Unit for Recording Erythrocyte Sedimentation Rates. J. W. CUTLER.

Lack of a universal technic has been a serious stumbling block to more widespread adoption of the blood sedimentation test in general practice. In every one of the technics described in the literature the unit of comparison (usually the drop at the end of one hour expressed in millimeters), while it reflects the rapidity of sedimentation in a general way, fails to give an accurate idea of the rate of settling. Furthermore, it also reflects anemia in the packing of the cells in the bottom of the tube within the first hour in rapidly settling blood.

The object of the present technic is to determine the rate of settling of the rouleaux at a time when the rate is least influenced by the period of aggregation of the red cells into rouleaux, on the one hand, or their packing in the bottom of the tube, on the other. The essential features are as follows: Cutler sedimentation tubes of 1 cc. capacity are used, which are graduated into 50 mm. divisions, with 0 at the 1 cc. level. One tenth of 1 cc. of a 3.8 per cent sodium citrated solution and 0.9 cc. of blood obtained by puncture of a suitable vein are gently mixed in a 2 cc. syringe and poured into the sedimentation tube, and the tube is placed in a special rack. The position of the sedimenting column of erthrocytes is determined every five minutes for one-half hour and recorded on special charts.

The biggest drop in any five minute period during the first half hour is the maximum sedimentation rate in five minutes (M.S.R.) that the rouleaux will attain in the Cutler tube, no matter how many readings are taken. This maximum rate becomes the unit of comparison.

A maximum settling of 1 mm. or less in five minutes during the first half hour is normal. Anything more than this is abnormal and therefore pathologic. A maximum rate of 1.5 to 3.5 mm., although pathologic, is usually not associated with constitutional manifestations of disease such as a fever or a rapid pulse. In such instances the pathologic process responsible for the increased rate is usually latent or subclinical. A maximum sedimentation rate of 4 to 9.5 mm. in five minutes, on the other hand, is usually associated with constitutional symptoms, but these are of mild to moderate severity. A maximum rate of 10 mm. or more in five minutes is almost invariably associated with marked constitutional symptoms.

This is all there is to a scientific determination and interpretation of the sedimentation rate. This unit—maximum sedimentation rate in five minutes (M.S.R.)—needs no correction for anemia. It cannot be misunderstood, for it means just what it says. It is important to remember that the "M.S.R. unit" is different in tubes of different length.

J. H. CLARK, *President*

H. L. RATCLIFFE, *Secretary*

Regular Meeting, Feb. 8, 1940

Nature, Incidence and Duration of Hodgkin's Disease. E. B. KRUMBHAAR.

The substance of this address can be found in the author's paper "A Symposium on the Blood and Blood-Forming Organs," published by the University of Wisconsin Press, Madison, Wis., 1939.

Idiopathic Hypertrophy of the Heart in Infancy. IRVING J. WOLMAN.

The cause of idiopathic cardiac hypertrophy is not known. Though rare at all ages, the disorder is encountered most frequently in infancy. Clinical and pathologic data on 5 patients are presented; these were infants aged between 4 and 18 months, who had died of sudden, unexpected circulatory failure. In none had the presence of heart disease been suspected during life. The hearts were dilated but were free from anomalies and from evidences of rheumatism and diphtheritic intoxication. The musculature was diffusely hypertrophied; each heart weighed from two to three times more than normal. In 4 of the 5 specimens wandering cells, chiefly mononuclears, were discovered within the interstitial connective tissue. The cause of the cardiac changes was not apparent; stains for glycogen showed no excess. Aerobic and anaerobic cultures of the ventricular muscle of 3 of the hearts were negative. The infants had not been anemic or syphilitic. It is possible that some obscure nutritional deficiency lies at the basis of this puzzling disorder. A review of the histories of the feeding of these infants shows that 4 of them had been receiving inadequate quantities of vitamin D in their daily diets; 2 had florid rickets at the time of necropsy. No other clues as to the causes were obtained.

Book Reviews

Architecture of the Kidney in Chronic Bright's Disease. Jean Oliver, Professor of Pathology, Long Island College of Medicine, Brooklyn. Cloth. Pp. 257, with 112 illustrations. Price $10. New York and London: Paul B. Hoeber, Inc., 1939.

This impressive volume comes at an opportune time, for it brings refreshment of mind and valuable information to pathologists who have long been exasperated by the flow of petty additions to the histologic details of diseased kidneys. The reviewer, for one, is grateful to the author and to the Josiah Macy Jr. Foundation, which has supported his studies and made possible this publication.

The book deals with the study of diseased kidneys by microdissection and reconstruction methods, supplemented and controlled to some extent by ordinary histologic studies.

A rapid perusal of the book gives one the impression of a vast amount of painstaking work, done honestly and presented on the whole dispassionately. As might be anticipated, the number of details encountered in these studies was great and the difficulties of presentation correspondingly difficult. The author is to be complimented on his manner of approach and his success in exposition.

Rereading, with careful consideration of the author's discussions and such correlations as he makes with what is known about the pathologic physiology of the kidney, convinces the reviewer that significant new facts are established and that the importance of the volume is great because of the stimulus it provides for the imagination. The illustrations throughout the book are superb and achieve their purpose. A number have been taken from previous studies of Oliver and his collaborators, published in the ARCHIVES OF PATHOLOGY in 1933, 1934 and 1935, but these are few in comparison with the additions.

The first chapter is devoted to "Technique and Methods. Material and Method of Presentation."

The second, third and fourth chapters present objectively the structural changes encountered in glomeruli and tubular systems in the various forms of Bright's disease.

Chapter 2 is concerned with the two outstanding features of chronic kidney disease, hypertrophy of the nephron (glomerulo-tubular unit) and atrophy of the nephron. Results are illustrated from reconstructions.

Chapter 3, "The Morphology of the Abnormal Nephron," describes the glomerulus and various parts of the tubules as encountered in terminal Bright's disease. Most of the examples were taken from glomerular nephritis; a few, from arteriosclerotic nephrosclerosis. The purpose of the chapter is to present the complete range of abnormalities found, with emphasis on the fact that the typical morphologic changes are the same in all forms of the renal lesion. The many illustrations are from photographs of microdissections.

Chapter 4 is entitled "The Aglomerular Nephrons of Terminal Bright's Disease" and establishes the fact that tubules may persist after destruction of their glomeruli. Good evidence is presented that such tubules are functional. The illustrations are from microdissections and reconstructions and fully document the author's conclusions.

Chapter 5 is a short one, entitled "The Transformation of the Arterial System in Terminal Bright's Disease." It is illustrated chiefly from microdissections. It demonstrates the formation of new vascular channels which serve to make the tubules independent of the passage of blood through the glomerulus. The vessels

are (1) Ludwig's vessel, which arises from the afferent arteriole and breaks up into the intertubular network, (2) a branch which develops from the interlobular artery and ends in the intertubular capillaries and (3) branches which arise from the arcuate artery or the deep portion of the interlobular artery and descend in the medulla. Such descending arteries may also take origin from Ludwig's vessel.

The sixth chapter, "Factors Responsible for the Regressive Alterations of the Kidney Parenchyma in Terminal Bright's Disease," discusses destruction of glomeruli as a cause of tubular atrophy and arterial disturbances responsible for nephron regression. These two parts of chapter 6 are informative and convincing. The third part of chapter 6—"The Interstitial (Inflammatory) Reaction As It Affects the Parenchymal Elements"—is the weakest part of the book and perhaps is the only part which can be considered to provide bait for disputative-minded pathologic histologists. It is the only part in which the author has indulged to a slight degree in interpretative comments—something which he warmly deprecates in his consideration of much of the current pathology of the kidney. On the whole, the reviewer thinks it is somewhat regrettable that the word "inflammatory" has been used in regard to the behavior of the interstitial tissue of the kidney in view of the different connotations aroused by that term in the minds of thoughtful pathologists. However, part 4 of this chapter, devoted to "Obstructive or the Hydronephrotic Factor in Nephron Distortion," again brings objective details valuable as premises for thoughtful speculation.

Chapter 7, in which the author takes up "The Pattern of Kidney Structure in Bright's Disease," is well presented and illustrated by a selection of objective findings of great beauty, obtained from cases studied and presented in the book by Adöis and Oliver, published in 1931, entitled "The Renal Lesion in Bright's Disease" (Paul B. Hoeber, Inc., New York). The general plan is to present microdissections on one page and a histologic section on the opposite page, with a brief résumé of the clinical history of the patient and the histologic observations. The author has taken great pains and has shown ingenuity in the use of devices in his illustrations in order to give information concerning details of pathologic alteration of the nephron and the blood vessels.

There is a final chapter—the eighth—entitled "Epicrisis and Prolegomenon"—which consists of only 9 pages in which the author expresses rather more optimism in regard to the possibility of correlating the details he describes with function. Rightly, he insists that further advances in knowledge require collaboration by pathologists and clinicians competent in physiologic methods of study. Perhaps one might say that occasionally he goes a bit too far in assuming that alterations in structure are indicative of corresponding qualitative changes in function.

A third reading of the book has convinced the reviewer that wherever the author has exposed himself to adverse criticism there is occasion only for sympathetic and respectful toleration, because it is evident that in the performance of such a large amount of work many observations must have been made (known only to him) that are not now amenable to analysis and presentation.

To review this book adequately would require a great deal of space, and it is doubtful if it can be done satisfactorily without reproduction of some of the illustrations. The reviewer believes that the material is so beautifully and clearly presented that well informed students of nephritis can make use of it for their own purposes and may very well come to conclusions different from those expressed by the author.

The actual contributions that Oliver has made are extraordinarily impressive: (1) the demonstration that tubules persist and apparently are functional after the glomerulus is completely destroyed; (2) that tubules with intact glomeruli, as well as aglomerular tubules, undergo hypertrophy; (3) the demonstration that in the course of nephritis important circulatory changes take place in the kidney—for example, the development of Ludwig's arteriole and the establishment of other arterial supplies to the tubular network from the interlobular arteries; (4) the

varieties of morphologic change that may occur in the tubule of a single nephron; (5) the obstructive role of material within the tubule, and (6) the change in the pattern of the blood supply in vascular nephritis.

All pathologists interested in diseases of the kidney or forced, in their routine, to write descriptions of diseased kidneys should have this book. The reviewer has already derived great satisfaction in his better understanding of microscopic slides of diseased kidneys. The physiologically trained clinical investigator of nephritis will also find this book of value. It will make clear to him the difficulties of the pathologist and should provide some new ideas for methods of study during life.

Dr. Oliver makes no claim for contributions on the genesis of Bright's disease, nor is it apparent that the methods he has used are adapted for this objective. The importance for physiology through encouragement of research on the physiology of the tubule is apparent. While there is some doubt in the mind of the reviewer of the probability of bringing to light by these methods other facts approaching in importance those already presented by Oliver, he has no hesitancy whatever in proclaiming this book to be outstanding in importance, one that will materially influence the trend in investigations of the physiology and pathology of the kidney.

Electrocardiographic Patterns. Arlie R. Barnes, M.D., The Mayo Clinic, Rochester, Minn. Cloth. Pp. 197, with 94 illustrations. Price $5. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

This excellent book represents an extraordinary amount of clinical, experimental and pathologic work in electrocardiography since 1928. In the fifteen years which have elapsed since the publication in 1925 of Sir Thomas Lewis', "The Mechanism and Graphic Registration of the Heart Beat," one can only be amazed at the tremendous amount of new material which Barnes (and other workers in this field, largely Americans) have contributed. Barnes, working with Whitten in 1928, "became interested in the possibility of working out correlations of electrocardiographic changes with specific pathologic conditions in the heart." Within certain limitations and with occasional exceptions the author believes, and this reviewer believes, they have succeeded, thus fulfilling Herrick's prophetic question of 1919: "May it perhaps be possible to localize a lesion in the coronary system with an accuracy comparable to that with which we locate obstructive lesions in the cerebral arteries?"

The book opens with a clear description and with appropriate drawings of "the relation of the distribution of the coronary arteries to acute myocardial infarction," showing that, contrary to widespread belief, infarction occurs with about equal frequency in the anterior-apical and posterior-basal portions of the left ventricle, although occlusion of the anterior descending branch of the left coronary artery occurs more commonly than does occlusion of the right coronary artery. Barnes emphasizes that the resulting electrocardiographic pattern reflects the *site* of *infarction* of the left ventricle (not the *vessel* occluded), and later he offers certain explanations as to the extraordinary infrequency of infarction of the *right* ventricle.

Chapter 3 discusses the "electrocardiogram in acute myocardial infarction and in its healing stages," differentiating the so-called Q_1T_1 patterns from the Q_3T_3 patterns, etc. One important comment is made regarding the value of serial electrocardiograms in the diagnosis of acute and healing myocardial infarction, namely, that other factors, such as "acute pericarditis, acute cor pulmonale, diabetic acidosis, digitalis therapy, certain acute infections, may likewise produce a tracing which differs from day to day."

In contrast to the usefulness of the electrocardiographic patterns in the diagnosis of myocardial infarction, attention is called to "the role of the electrocardiogram in the diagnosis of *nonocclusive* coronary disease." Noting that while many patients with advanced coronary sclerosis, even when it results in myocardial

fibrosis, have normal electrocardiograms, "organic delay in auriculoventricular conduction, and complete and incomplete bundle branch block are associated in a high percentage of cases with nonocclusive coronary sclerosis."

The electrocardiographic pattern of predominant strain of either ventricle, manifested at autopsy by ventricular hypertrophy and dilatation and during life by failure of the left or of the right side of the heart, is discussed in chapter 4. The electrocardiogram in *acute* right ventricular strain (acute cor pulmonale) is described in chapter 5, with particular reference to the role of pulmonary embolism and infarction as a classic example of unilateral ventricular strain. Chapter VI discusses the electrocardiogram in *chronic* right ventricular strain.

The electrocardiogram in pericarditis is considered in chapter VII, with many new contributions to present knowledge. One particularly interesting statement may be noted: "It is therefore obvious that the weight of evidence derived from experimental and clinical observation indicates strikingly that the electrocardiographic changes in pericarditis, in most instances at least, are an expression of the associated subepicardial myocarditis."

The book closes with chapters on the "effects of certain drugs, metabolic disorders and infections on the electrocardiogram," and "some observations relative to precordial leads." One feels that with the extensive references to pertinent literature, the complete author and subject index, the fine format of the book itself and the wide experience and sound clinical judgment of the author, this book constitutes a noteworthy contribution to an important and ever widening clinical and physiologic discipline.

Books Received

THIRTY-SEVENTH ANNUAL REPORT, 1938-1940, OF THE IMPERIAL CANCER RESEARCH FUND. Founded under the direction of the Royal College of Physicians of London and the Royal College of Surgeons of England, 1902. Incorporated by Royal Charter, 1939. Paper. Pp. 43. London: Taylor and Francis, 1940.

THE FIFTY-FIFTH ANNUAL MEDICAL REPORT OF THE TRUDEAU SANATORIUM AND THE THIRTY-FIFTH MEDICAL SUPPLEMENT FOR THE YEAR ENDING SEPTEMBER 30, 1939, TOGETHER WITH THE TWENTY-THIRD COLLECTION OF THE STUDIES OF THE EDWARD L. TRUDEAU FOUNDATION FOR RESEARCH AND TEACHING IN TUBERCULOSIS, 1939.

THE SARANAC LABORATORY FOR THE STUDY OF TUBERCULOSIS OF THE EDWARD L. TRUDEAU FOUNDATION: REPORT OF THE DIRECTOR AND FINANCIAL REPORT FOR THE YEAR ENDING SEPTEMBER 30, 1939. REPRINTS OF SCIENTIFIC PAPERS. Saranac Lake, N. Y.: The Saranac Lake Academy of Medicine, 1939.

CARE OF POLIOMYELITIS. By Jessie L. Stevenson, A.B., R.N., Consultant in Orthopedic Nursing, National Organization for Public Health Nursing. Cloth. Pp. 230, illustrated. Price, $2.50. New York: The Macmillan Company, 1940.

YOUTH LOOKS AT CANCER. A Text Prepared for Colleges, Preparatory Schools and High Schools. The Westchester Cancer Committee, Bronxville, New York. Cloth. Price, 75 cents. Pp. 55, illustrated. Brookville, N. Y.: Brookville, Press, 1940.

BIOLOGICAL SYMPOSIA. Volume 1. Edited by Jaques Cattell, Editor of the American Naturalist and American Men of Science. Foreword by Albert F. Blakeslee, Director of the Department of Genetics, Station for Experimental Evolution of the Carnegie Institution of Washington. Cloth. Pp. 238, illustrated. Price, $2.50. Lancaster, Pa.: The Jaques Cattell Press, 1940.

INFORME DE LA JUNTA DE BENEFICENCIA DEL DISTRITO FEDERAL. CORRESPONDIENTE A SUS ACTIVIDADES EN EL LAPSO COMPRENDIDO DEL 1 DE DICIEMBRE DE 1938 AL 30 DE NOVIEMBRE DE 1939. Paper. Pp. 166, illustrated. Caracas, Venezuela: Tipografia la Nacion, 1940.

A TEXTBOOK OF PATHOLOGY. W. G. MacCallum, Professor of Pathology and Bacteriology, Johns Hopkins University, Baltimore. Seventh edition, thoroughly revised. Cloth. Pp. 1,302, with 697 illustrations. Price, $10. Philadelphia: W. B. Saunders Company, 1940.

PATHOLOGICAL HISTOLOGY. Robertson F. Ogilvie, M.D., F.R.C.P. (Edin.), Lecturer in Pathology, University of Edinburgh. Foreword by A. Murray Drennan, M.D., F.R.C.P. (Edin.), Professor of Pathology, University of Edinburgh. Cloth. Pp. 332, with 220 illustrations in color. Price, $8.50. Baltimore: Williams & Wilkins Company, 1940.

DYNAMICS OF INFLAMMATION. AN INQUIRY INTO THE MECHANISM OF INFECTIOUS PROCESSES. Valy Menkin, Department of Pathology, Harvard Medical School. Experimental Biology Monographs. Cloth. Pp. 244, with 50 illustrations. Price, $4.50. New York: The Macmillan Company, 1940.

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VOLUME 29 NUMBER 6

ARCHIVES OF PATHOLOGY

JUNE 1940

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, $8.00

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of Congress of March 3, 1879

THE ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance pathology in the United States and to promote research and observation in this field.

Manuscripts for publication, books for review and correspondence relating to contributions should be sent to Dr. Ludvig Hektoen, Chief Editor, 629 South Wood Street, Chicago, or to any other member of the Editorial Board. Communications regarding subscriptions, reprints, etc., should be addressed, ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago.

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